Original Research Article

Subjective and objective interpretation of tear film interferometry images

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ABSTRACT

Background: Assessment of the tear film is necessary in routine clinical practice because an unstable tear film can hamper the quality of life by causing vision-related problems and compromising the ocular surface. One of the major concerns related to an unstable tear film is dry eye. Many of dry eye patients suffer from a lack of meibum which forms the lipid layer of the tear film. The lipid layer can be graded and interpreted by using interferometry. However, interpretation and grading of this dynamic layer may be inconsistent in terms of inter- and intra- observations. This study investigated the difficulty of consistent, subjective grading of clinical findings, in general.

Methods: The interferometry images of 30 subjects captured from different equipment were analyzed subjectively. The agreement between intra-observer repeatability was also measured.

Results: A positive Spearman’s correlation of 0.81 was found among different grading patterns observed using the Tearsope to compare right and left eyes. Similarly, a positive Spearman’s correlation of 0.63 was found among different grading patterns observed under interferometer in right and left eye. Correlations were statistically significant, p<0.001. The agreement between intra-observer repeatability calculated using Cohen’s kappa values were also statistically significant, p<0.001.

Conclusions: A correlation between the findings of different equipment could not be made due to the differences in wavelengths of incident light and the image details. However, a new grading pattern has been proposed to describe the thickness of various lipid layer patterns observed under Doane’s interferometer.

Keywords: Dry eye syndrome, Lipid layer pattern, Tear film lipid layer

INTRODUCTION

The tear film, or the precorneal film as described by Wolff, is composed of three layers - an anterior lipid layer, a middle aqueous layer, and a posterior mucus layer.1 Most of the tear film is secreted by the lacrimal glands, under the influence of the nervous system.2 The structure of the precorneal film is continuously changing because of the tear flow, evaporation, and blinking.3 The reported thickness of the pre-corneal film appears to be dependent on the test used, resulting in values that vary between 5µm to 46µm, whereas the reported volume ranges less dramatically from 3µl-10µl in normal subjects.4,5 The tears are evenly distributed over the ocular surface via blinking.6 This blinking mechanism is highly variable and can be affected by physiological and psychological factors.7 The Lipid layer is the outer most layer of the tear film and is made up of lipids which is sometimes also referred as Tear Film Lipid Layer (TFLL).8 The primary role of the TFLL is to prevent the evaporation of the underlying aqueous layer and this has been a topic of interest in several studies.9-10 Evaporation
of the tear film is directly associated with the thickness of the TFLL.\textsuperscript{11} Work done by Peng and colleagues found a four-fold increase in the evaporation rate in subjects with no detectable TFLL as compared to those with a normal lipid layer.\textsuperscript{12} The quality and quantity of the lipids can help eye care professionals to understand the mechanism of dry eye syndrome (DES).\textsuperscript{13} According to the Tear Film and Ocular Surface Society (TFOS) DEWS II, dry eye is defined as:\textsuperscript{14}

“A multifactorial disease of the ocular surface characterised by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.”

Evaluation of the tear film is important for both clinicians as well as researchers. Stability of the tear film provides evidence to clinicians, helping them to make their diagnosis and monitor the effect of treatment. It is also useful to researchers in investigating the surface properties of newer contact lens materials.\textsuperscript{15} Like DED, there is no gold standard diagnostic test to evaluate the tear film, yet there are several ways of determining the quantity and quality of the tear film.\textsuperscript{16} One of the non-invasive methods is Interferometry. Doane developed a specialised interferometer to observe real time interferometric tear film images.\textsuperscript{17} Another non-invasive piece of equipment was developed by Guillon to observe the structure and behaviour of the tear film.\textsuperscript{18} Both these tools depend on the observation of wave characteristic of reflected light observed from the anterior and posterior surface of the lipid layer.

METHODS

The study was conducted at Glasgow Caledonian University Scotland, United Kingdom. Ethical and legal practices were followed in accordance with World Medical Association Declaration of Helsinki.\textsuperscript{19} The confidentiality and anonymity of all the participants has been maintained. The School of Health and Life Sciences granted the ethical approval for this study. Participants were recruited by a word-of-mouth and e-mails through personal and mutual acquaintances within Glasgow. Subjects under the age of 18 years and subjects wearing contact lenses were excluded from the study. The Ocular Surface Disease Index (OSDI) and the McMonnies’ dry eye questionnaires were used as screening tools to grade the severity of dry eyes.\textsuperscript{20,21}

Image Acquisition-Interference images were, first, observed using a Tearscope-plus which was attached to a specially modified slit-lamp biomicroscope and viewed at magnification of 10x. Due to the self-illuminating nature of the Tearscope, the room lights were switched off to avoid any artefacts. The exposure level on the capturing device was readjusted to ensure a clear pattern of fringes was seen. Participants were asked to blink normally during the entire session. To capture the image a smartphone (Lenovo Moto G4 plus) with a 16-megapixel, f/2.0, phase detection and laser auto focus camera was used. Multiple images of both eyes were taken.

Using the same slit-lamp, indirect illumination was used to visualize the interference fringes, without the benefits of Tearscope illumination. These were taken using the same smartphone, in order to facilitate direct comparison of methods. The final set of interferometric images were captured using the Doane’s interferometer. Images were captured with a help of a Euromex® CMEX-1301 camera attached to the Doane’s interferometer. This CMEX-1301 camera was connected to a computer. Multiple Images of both the eyes were captured via Euromex’s ImageFocus software. Due to the heterogeneity of the lipid layer it was necessary to select a region of interest (ROI) while grading the interferometric images acquired from Tearscope. This ROI was selected from in front of the lower part of the iris because of a better contrast.\textsuperscript{22,23} Images from the Tearscope were observed and graded into the categories described by Guillon.\textsuperscript{24} Interference images from Doane’s interferometer were also graded into the categories described by Thai and Remeseiro.\textsuperscript{25} The Image evaluation was done in three sessions by the same observer. In the first session, the images were graded to familiarize the observer with the grading system. The findings from the second and third sessions were used to calculate the intra-observer variability.

To estimate the thickness of different interference patterns, image processing software was used (ImageJ). Data analysis was done on MS-Excel, IBM-SPSS (version 23), and ImageJ (www.imagej.nih.gov).

RESULTS

A total of 30 subjects (n=30) comprised of students and lectures aged 18 to 55 (mean age 34±10 years) from Glasgow Caledonian University participated in study. The participants were of mixed ethnicity (Caucasians, Africans, and Asians). The study comprised equal number of males (n=15) and females (n=15). According to the OSDI scoring (mean = 26.37±21.46), 27% of the subjects were normal while 33% had mild, 20% had moderate and 20% had severe dry eyes. And according to the McMonnies’ scoring (mean = 6.30±4.26), 80% of the subjects were normal while rest 20% had marginal dry eyes. The wave pattern was found to be the more common pattern (47% and 58% in right and left eye respectively) of the lipid layer images seen on Tearscope. While the coloured pattern was uncommon (17% and 10% in right and left eye respectively). Similarly, strong fringes without colours were more common patterns (47% in right and left eye) seen on Doane’s interferometer while, strong fringes with colours were uncommon (13% and 10% in right and left eye respectively).
To correlate the tear film grading patterns in the right and left eye in the same equipment, a non-parametric correlation (Spearman’s rho, 2-tailed) was used. A positive correlation of 0.81 was found among the patterns observed under Tearscope in right and left eye. Similarly, a positive correlation of 0.63 was found among the patterns observed under Doane’s interferometer in both eyes. All the correlations were statistically significant p<0.001.

Further a correlation between OSDI and McMonnies’ dry eye questionnaire was made using the same non-parametric test (Spearman’s rho, 2-tailed). The Correlation was statistically significant p<0.001.

Cohen’s kappa coefficient measured the Intra-observer repeatability. Cohen’s kappa values are classified as follows- <0.00, poor; 0.00-0.20, slight; 0.21-0.40, fair; 0.41-0.60, moderate; and 0.61-0.80, substantial; A value of more than 0.80 is described as close to perfect.26,27 All the kappa values were statistically significant p<0.001.

DISCUSSION

The lipid layer plays an important role in maintaining a stable tear film. Interferometry is one of the non-invasive methods which is used to study the lipid layer and has been practised since the late 80s. Work done by Guillon, Norm and Mcnald showed the use of interferometry in observing the lipid layer.28-30 When light, travelling in one medium (M1), is incident on another medium (M2), it may be reflected or transmitted, the proportion of these being dependent on relative refractive indices of M1 and M2, and angle of incidence. If light continues through M2 and meets another medium (M3), the division between reflection and transmittance can occur again. In case of a thin film, the path lengths for a ray, that has been split, and reflected by the front surface and that of a ray reflected by the posterior surface will only be slightly different, and can recombine, producing interference. Based on the relative phase shift of the reflected light, bright fringes are formed in constructive interference (in phase) and dark fringes are formed in destructive interference (out of phase). Monochromatic light gives rise to monochromatic fringes whereas, white light produces coloured fringes. Thicker lipid layers are more easily observed as they show stronger coloured patterns along with other distinct morphological features whereas, thinner lipid layers are difficult to observe due to lack of any such features. Patterns with same appearance/colour correspond to the same thickness. Guillon in 1998 used the Tearscope to evaluate the lipid layer, proposing five main grades of lipid layer interference patterns. Open meshwork, Closed meshwork, Wave, Amorphous, and Colours. The original interferometric grading system, developed by Doane and later modified by Thai and co-workers, proposed five grades of the pre-corneal film structure.25 According to their classification which was based on the observation of the tear film appearance, lack of fringes corresponded to a thinner lipid layer, uniformly spread fringes corresponded to a balanced lipid layer, while other patterns primarily focused on mixing and non-mixing of lipids with a characteristic appearance of “Islands”. While developing an automated technique to grade the lipid layer, Remeseiro and co-workers modified the original grading system. These modifications were necessary due to the differences in details observed in images which were captured by different digital cameras (Euromex© CMEX-1301). Based on the observation of contour lines, and appearance of grey and coloured fringes, they described the lipid layer as having Strong fringes, coalescing strong fringes, Fine fringes, coalescing fine fringes, and Debris.

Figure 1: Image graded as fine fringes. ROI covers approximately 90% area of the interferometric fringes.

Figure 2: Surface plot when ROI covers 90% area of interferometric fringes.

In this study, to estimate the thickness of the different interferometric patterns ImageJ’s Interactive 3D surface plot plugin was used in which the luminance of each pixel in the image is interpreted as the height for the plot.31 Using available resources, two methods were applied to estimate the thickness of the lipid layer. For the first method, the ROI was selected such that approximately 90% of the area in each image could be
selected (Figure 1 and Figure 2). This was achieved using an elliptical selection tool. All the images were processed in RGB colour formats. This method however had to be rejected as it resulted in huge overlaps in thickness levels among various grading patterns.

For the second method (Figure 3 and Figure 4), the ROI was selected at the area where distinct/clear interferometric fringes were observed. This reduced the chances of uncertainty as several other unwanted features including blurred areas were rejected.

- Strong fringes without colour: A pattern with an appearance of dark and light bands of grey fringes, without appreciation of any coloured fringes.
- Strong fringes with colours: A pattern with an appearance of dark and light bands of grey fringes, with appreciation of one or more coloured fringes.

**Table 1: New interference grading patterns.**

<table>
<thead>
<tr>
<th>Image</th>
<th>Pattern</th>
<th>Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak fringes</td>
<td>20~30 nm</td>
<td></td>
</tr>
<tr>
<td>Fine fringes</td>
<td>30~50 nm</td>
<td></td>
</tr>
<tr>
<td>Strong fringes</td>
<td>40~90 nm</td>
<td></td>
</tr>
<tr>
<td>Strong fringes</td>
<td>60~110 nm</td>
<td></td>
</tr>
</tbody>
</table>

Doane, and Thai in their respective studies on lipid layer interference patterns has mentioned that these LLPs are analogous to topographical contour maps and can provide detailed information about the thickness of the lipid layer.17,25 The lipid layer thickness range in this study (20-110nm) is homologous with the recently reported thickness range of the lipid layer between 15-157 nm by TFOS DEWS-II.32 The overlapping range of thickness in different patterns as described in this study are probably

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**Figure 3: Region of interest selected as area of clear fringe appreciation.**

**Figure 4: Surface plot when a particular ROI is selected.**

Using the second method, the thickness range of each pattern was calculated, and four new patterns were developed (Table 1).

- Weak fringes: A pattern with no or hardly any noticeable fringes.
- Fine fringes: A wavy pattern with an appearance of delicate grey fringes.
due to the non-uniform nature of the lipid layer. With closure of the lids, the lipids are released from the Meibomian glands and due to the sheer force of upper eye lid these lipids are pulled and distributed over the ocular surface. Most of the lipids remain concentrated at the lower lid margin and their concentration decreases at the upper lid margin. The non-uniform mixing of lipids makes several areas thick and thin. This non-uniformness is due to the difference in compositions and melting points of lipids.\(^2\) According to the results of this study more than half of the subjects graded as severe dry eye on OSDI scoring were found to have a lipid layer \(\geq 30\)nm. Similar results were reported by other researchers when they observed a thicker lipid layer in ADDE.\(^33,34\) This was probably due to the failure of lipid spread in the absence of adequate aqueous film.

A strong and moderate correlation among the different grading patterns in right and left eye observed under Tearscope and interferometer respectively can be explained at neuro-sensory level. A stimulus to the ocular surface, simultaneously activates the sympathetic and parasympathetic nerves, which stimulates the secretion of tears from the lacrimal glands in both eyes.\(^32\) It is important to note that, lacrimal glands secrete the aqueous component of the pre-corneal film. The difference in appearance is however due to the uneven spreading of the lipids which results in considerable variations in the patterns that are observed.

This study also reported a moderate correlation between the dry eye questionnaires. This is perhaps due to the difference in the nature of questions and their responses used to grade the severity of dry eyes. The OSDI focuses on the symptoms, visual functions, and environmental influence on these symptoms. Whereas, the McMonnies’ focuses on the sensitivity of environmental factors and associated systemic conditions that can influence dry eye.

The thickness range of Guillon’s classification observed on Tearscope does not correspond to the new thickness range of different patterns observed under interferometry as reported in this study. This is probably due to the differences in the wavelengths used to illuminate the ocular surface. A similar concept was mentioned by Thai and colleagues where they stated that a comparison among the grading patterns acquired from different interferometers cannot be made.\(^22\) This is probably due to the different composition of spectrum or different distribution of wavelengths. Also, there is a considerable difference in the area illuminated by the both the apparatuses. Furthermore, image details vary highly when observed under these apparatuses.

Images observed under the slit-lamp showed the appearance of spots/particles and other artefacts and hence these images were not graded due to the lack of any specific patterns and other morphological features.

Although, this study estimates the thickness of the lipid layer patterns observed under Doane’s’ interferometer, exact thickness can only be measured using more sophisticated equipment like spectrophotometers and colorimeters. The thickness of the lipid layer can vary during the interblink periods, and post-blink a completely different pattern can be observed. Furthermore, interpretation and grading of this dynamic layer is highly subjective and inconsistent when repeatability is concerned, in terms of inter- and intra- observations.

**CONCLUSION**

Evaluating the lipid layer patterns can give an idea regarding the stability of the tear film because a thinner lipid layer is associated with faster influence on evaporation. Even after more than four decades of continuous research, certain discrepancies are still there about the thickness of the tear film. This study proposed a new grading system which can be a further step for eye care professionals in evaluating the lipid layer and understanding the mechanism of DED. Due to the highly subjective nature of lipid layer grading some amount of uncertainty and misinterpretations are always there.

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