Introducing a New Parameter for the Assessment of the Tear Film Lipid Layer

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Purpose. The differential diagnosis of dry eye syndrome is still a challenging task. The purpose of this study was to understand the relationship between a novel, objective clinical parameter, the "corrected lipid layer stabilization time," and commonly performed clinical tests for dry eye patients.

Methods. Data were obtained from a prospective clinical study with 59 patients of different subjective severity, as determined with the Ocular Surface Disease Index (OSDI). The dynamics of the tear film lipid layer were made visible through a white light source and were stored digitally. Because the distance between the upper and lower eyelid affects the lipid layer dynamics and varies significantly between subjects, the distance of the eyelids was determined and used to correct the lipid layer stabilization time. The resulting parameter was compared with common clinical procedures.

Results. The corrected lipid layer stabilization time has a highly significant correlation with tear film breakup time (Spearman $r = -0.485$, $P < 0.01$), Schirmer test without anesthesia ($r = -0.431$, $P < 0.01$) and with the Ocular Surface Disease Index ($r = 0.498$, $P < 0.01$). It also correlates with the lissamine green staining score ($r = 0.379$, $P < 0.05$), but shows no correlation with the osmolarity of the tear film. Without the correction for the eyelid opening, the correlations decrease considerably.

Conclusions. These data suggest that the diagnostic value of the lipid layer stabilization time for the assessment of the severity of dry eye syndrome increases considerably when it is corrected by the distance of the eyelids. (*Invest Ophthalmol Vis Sci.* 2012;53:6638–6644) DOI:10.1167/iovs.12-10257

Some of the main purposes of the pre-ocular tear film are the maintenance of a proper homeostasis and the protection of the exposed ocular surface cells. To fulfill this function, the tear film is organized in three different layers: the innermost layer consists mainly of mucins secreted by ocular surface epithelial cells; the middle layer provides the aqueous solution for proteins and electrolytes and is secreted primarily by the lacrimal glands; the outermost layer, consisting mainly of nonpolar and polar lipids, seals the tear film. It thereby prevents the aqueous compartments from hyperevaporation.[1–3] In healthy subjects, the tear film is rebuilt with each blink and remains stable during the interblink interval.

Popular risk factors that harm the construct of the tear film include contact lens wearing,[4] working in air-conditioned environments,[5] visual display terminal usage,[6,7] and the hormonal status.[8] Additionally, Laser-Assisted in situ Keratomileusis (LASIK) surgeries[9] and autoimmune disorders, such as Sjogrens syndrome,[10] contribute to the destabilization process.

The diagnosis of an insufficient tear film is often referred to as dry eye disease (DED) or keratoconjunctivitis sicca, which can be considered as a subgroup of ocular surface diseases.[11] It is defined as a "multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear instability with potential damage to the ocular surface. Dry eye is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface."[12]

Symptoms of discomfort, dryness, or grittiness soon occur if any of the previously described layers is lacking functionality.[13] Because the tear film is the first refracting structure for the incoming light, an inhomogeneous tear film is also often the reason for transient changes in visual performance after a blink.[14,15]

Due to the diversity of risk factors and etiology, as well as the complex interactions between the layers, the precise diagnosis of DED is challenging. A number of standard assessment procedures for tear film disorders have been developed and can be divided into subgroups: questionnaires to assess the subjectively reported symptoms; investigations of ocular surface damage via staining; the demonstration of the tear film instability by tear film break up time (TFBUT) as determined with fluorescein; and the lacrimal gland production capacity through the Schirmer test.[12] It is remarkable that these clinical investigations correlate quite poorly with the subjectively reported symptoms and vice versa.[13] This can be explained by the highly dynamic characteristics of the tear film and its sensitivity to the surrounding environment, as well as to the often invasively performed diagnostic procedures. Thus, the search for an objective, noninvasive but robust parameter for the diagnosis of DED is still of potential interest for both science and clinical routine. More recently, devices for measuring the tear film osmolarity have also been introduced, for example, Tearlab (Tearlab, Osmolarity System; Ocusense, San Diego, CA).

Besides those commonly performed investigations, a couple of noninvasively recorded parameters have been established to diagnose DED. Mengher et al. were among the first to record the noninvasive tear film break up time (NITBUT) by investigating the distortions of a pattern projected on the tear film.[16,17] Additionally, the tear film meniscus height[18] and the tear meniscus curvature[19] have been reported to give information on the quantity of the tear film. Nevertheless,

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commercially available devices for the assessment of these parameters are currently not widely distributed.

The importance of the outermost tear film lipid layer for the overall stability of the construct of the tear film was reviewed by Foulks et al.[20] They concluded that increased evaporation of the tear film, due to a compromised lipid layer, is one of the most common etiologies for the hyperosmolarity of the tear film and therefore highly responsible for the arising of keratoconjunctivitis sicca. Thus, a group of studies has focused on the investigation of the tear film lipid layer.[21,22] Goto and Tseng[22] have shown that the spreading time of the lipid layer after a blink is significantly prolonged in patients with DED compared with healthy subjects. These findings were extended by a second study on the lipid layer behavior, which investigated the dynamics of this outermost layer before and after a punctual occlusion.[23] This study reported that the lipid layer stabilization time was significantly reduced after this therapeutic procedure, indicating that the performance of the lipid layer is strongly influenced by the underlying aqueous tear fluid. Additionally, the lipid layer spreads more uniformly immediately after a blink in healthy eyes.

To further investigate this potentially useful parameter, we developed a novel device based on interferometry and compared the results with the standard diagnostic procedures for DED. Thus, the principal purpose of the current study was to find an objective, robust, and noninvasively assessable parameter for the evaluation of the tear film lipid layer that can be used for clinical routine. Because none of the currently common clinical dry eye tests objectively checks the functional status of the lipid layer, we believe that such a test would substantially enhance the diagnosis of patients with DED by providing a quantitative description of the specific performance of the tear film lipid layer.

Materials and Methods

Data were obtained from a prospective clinical study. Participants were chosen from the dry eye unit and from the staff of the Department of Ophthalmology, Medical University Graz. Subjects were excluded from the study if they showed symptoms of an active inflammation of the eye, any types of lid deformation, or if they had had ocular surgery within 6 months before the study took place. The experiments were approved by a local ethics committee and were in accordance with the 1964 Declaration of Helsinki. All subjects gave their informed consent to participate before their inclusion in the study. A total of 59 subjects ($n = 45$ female, $n = 14$ male) were included in the study. The subjects were divided into three subgroups, based on their subjectively reported severity as determined with the OSDI[24]: Subgroup 1 (mild, OSDI: 0–15, sex: 15/4 female/male, age: 55.2 ± 16.4 a), Subgroup 2 (moderate, OSDI: 16–30, 11/6 female/male, 52.9 ± 13.6 a), Subgroup 3 (severe, OSDI: 31–100, 19/4 female/male; 58.65 ± 13a). The distribution of the study population is shown in Figure 1.

Subjects were instructed not to use artificial tears or other local medications relevant to the tear film for at least 2 hours before the investigation. Contact lens wearers ($n = 5$) removed their contact lenses at least 8 hours before their examination. To characterize the subjects, tests were performed in the following sequence: OSDI, noninvasive lipid layer movement, osmolarity, TFBUT, lissamine green staining of the ocular surface, and Schirmer test (without anesthesia). All tests except the noninvasive evaluation of the lipid layer movement were performed on both eyes.

Lipid Layer Dynamics

To investigate the lipid layer dynamics, we developed a new diagnostic device (Fig. 2). This device illuminates the eye with a diffuse white light source, thereby making the lipid layer visible based on the physical principle of "white light interferometry," as previously described by Guillon.[25] Images were acquired via a USB microscope (PCE-MM200 Microscope; PCE Group, Meschede, Germany) and digitized as uncompressed audio–video interleaved (AVI) files at a spatial resolution of 640×480 pixels and at a temporal resolution of 20 frames per second, resulting in an interframe interval of 50 ms. The brightness was considered to be low enough, as no patients reported photophobic sensations during the investigation. The required certified medical device tests ensured that the illumination level was safe for the patients.

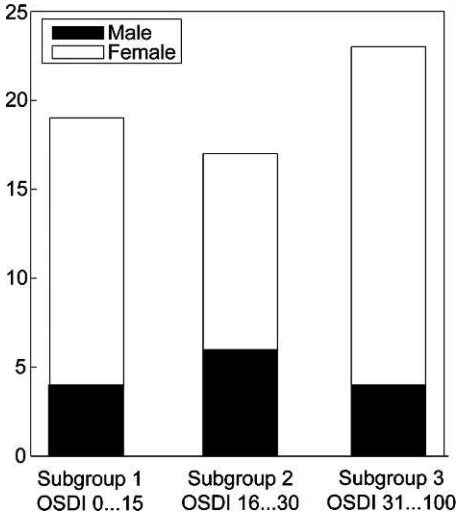

Figure 1. Distribution of the subjects.

The lipid layer stabilization time of five consecutive blinks was determined manually as the time between the beginning of the opening phase of the blink and the time when the movement of the lipid layer had stopped. The median value of five consecutive blinks was used for further analysis.

One representative image sequence of one subject is shown in Figure 3. Each subplot shows the interference image of the lipid layer. The first image is the onset of the opening phase of the blink; the last image represents the frame where no distinct movement of the lipid layer could be observed any more. The resulting, uncorrected stabilization time in this case was 2.1 seconds. Although the opening state of the eye has a direct influence on the tear film, we need some calibration parameter to correlate the distance between upper and lower eyelids, expressed in pixels, to the anatomical size of the eye. To achieve such a normalization, we chose to correct the lipid layer stabilization time by the ratio between the eyelid distance and the diameter of the iris. This compensates not only for differences in the size of the eye, but also for different distances between the camera and eyeball. Thus, the first step in our analysis was to fit a circle to the iris so as to extract the iris diameter. Subsequently, the distance of the eyelid margins was acquired through a parabola fit to each the upper and lower eyelid. The difference in vertical direction from the maximum value of the upper eyelid's parabola to the minimum of the lower eyelid's parabola was then taken as the eyelid distance in pixels (Fig. 4).

The measured lipid layer stabilization time was then corrected for the different eyelid opening states according to the following equation:

$$Tcorr[seconds] = Tmeasured[seconds] * \frac{IrisDiameter[pixels]}{LidDistance[pixels]}$$

Other Clinical Parameters

Tear osmolarity was measured with the TearlabOsmolarity System (Ocusense). A small sample of tear fluid was taken from the lower tear meniscus of each patient using a pen. The bottom of the tip thereby

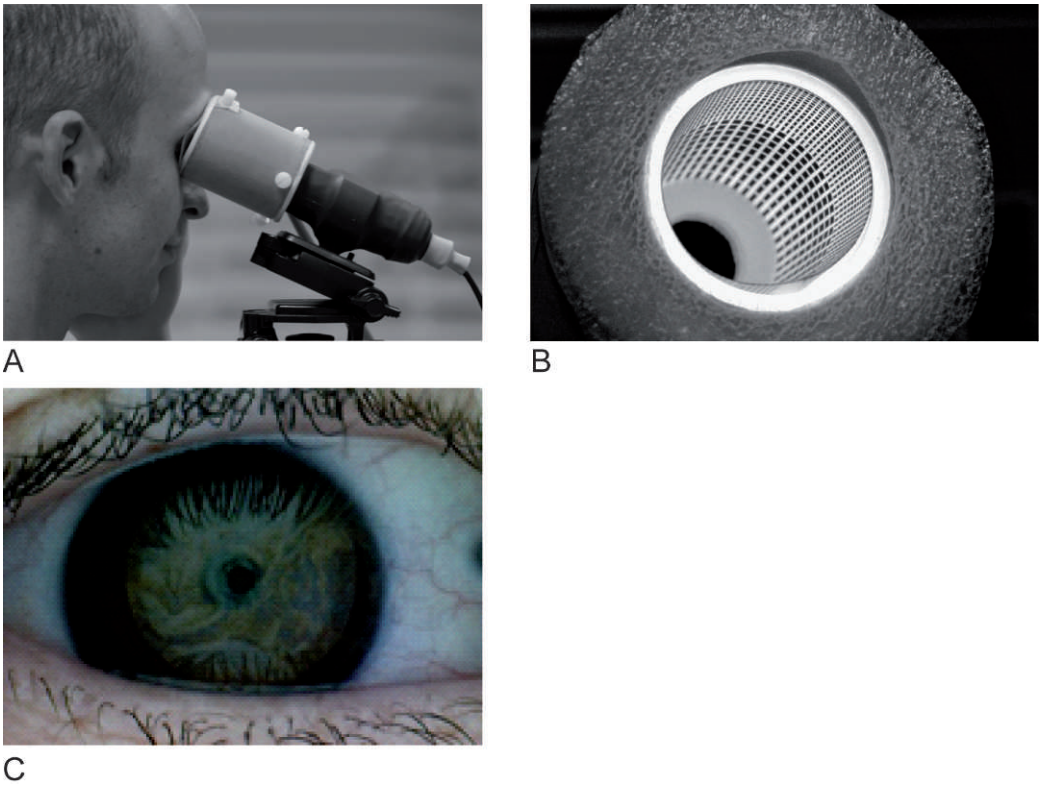

Figure 2. (**A**) The correct placement of the device during the investigation; (**B**) a close-up image of the opening of the device; (**C**) the resulting image.

came into contact with the thin line of moisture between the lower eyelid and the eye. Fluid was collected at the bottom tip of the test card and the result was displayed after a few seconds. The measurement range is linear from 275 to 400 mOsms/L.[12,26]

TFBUT was measured by touching the inferior temporal bulbar conjunctiva with a fluorescein sodium strip, wetted with a preservative-free isotonic sodium chloride solution. Patients were instructed to blink. The precorneal tear film was then examined under blue-light illumination using a biomicroscope (BQ900; Haag-Streit AG, Koeniz, Switzerland) with a 10-fold magnification. The mean value of a total of three measurements was recorded.

Sterile strips impregnated with lissamine green (HUB Pharmaceuticals, LCC, Rancho Cucamonga, CA) were used to classify the exposed interpalpebral portions of the nasal and temporal conjunctiva and the cornea. The extent of staining was graded according to the van Bijsterveld score of 0 to 3 (0, negative; 1, scattered minute; 2, moderate spotty; and 3, blotchy) for each zone, with a maximum score of 9.[27]

A 5-minute conventional Schirmer test without anesthesia was performed on closed eyes by placing a commercially available 5 × 35-mm paper strip (Haag-Streit, Harlow Essex, UK) over the lid margin at the junction of the middle and lateral third into the tear film.

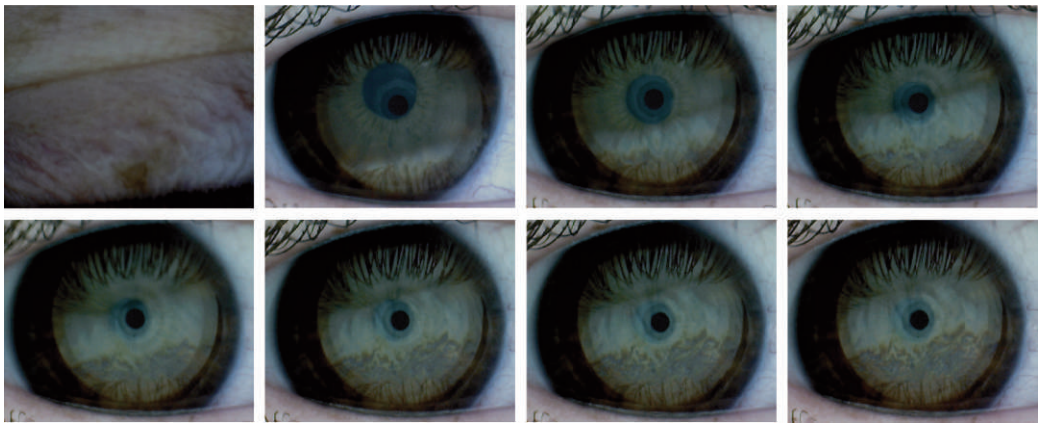

Figure 3. Representative image sequence from one subject. Starting from the opening phase of a blink sequence (*top left*), the lipid layer stabilization time was determined as the point where no visible movement could be observed any more (*bottom right*). The time difference between each of these images is 0.3 seconds, resulting in a lipid layer stabilization time of 2.1 seconds.

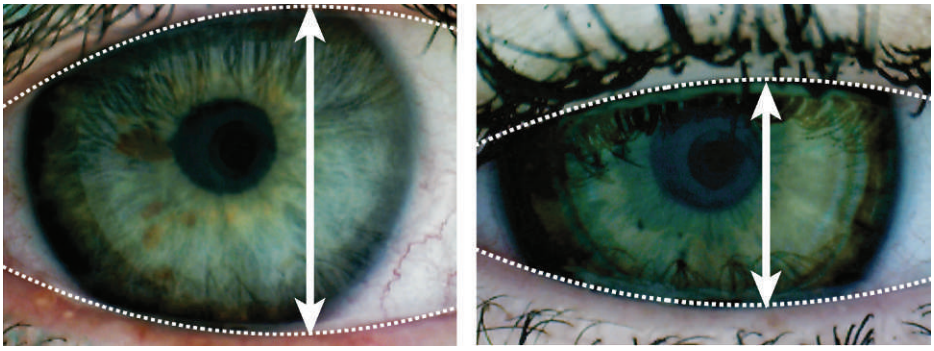

Figure 4. Large eyelid margin (*left*) compared with small eyelid margin (*right*) at the time of lipid layer stabilization.

Statistical Analysis

The correlation of the corrected and uncorrected lipid layer stabilization time with the other clinical parameters was determined through the calculation of Spearman's rank correlation coefficient. A linear regression analysis was conducted to quantify the relationship of the corrected lipid layer stabilization time and the other parameters.

Both the corrected and uncorrected lipid layer stabilization time, as well as the OSDI score, were checked for normality (Kolmogorov–Smirnov, $P > 0.05$; Q-Q-Plot). Because normality was not present, the nonparametric Wilcoxon signed rank test was used to check for significant differences between groups of different subjective severity. Significance level was set to $P < 0.05$ for significant correlations, and $P < 0.01$ for highly significant correlations. Statistical analysis was performed with the Statistics Toolbox of Matlab (Matlab; MathWorks, Natick, MA).

Results

The linear relationship of the corrected lipid layer stabilization time and the subjectively reported severity for all subjects is shown in Figure 5. The correlation coefficient between OSDI and the corrected lipid layer stabilization time was $R^2 = 0.43$, $R^2 = 0.28$ without the correction. The correlation between the OSDI and the other parameters are as follows: R^2 TFBUT: 0.12, R^2 Schirmer test: 0.09, R^2 corneal lissamine staining: 0.08, R^2 osmolarity: 0.22. The mean value for the uncorrected lipid layer stabilization times within the groups of different severity were 1.11, 1.83, and 2.12 seconds, the corrected lipid layer stabilization time within the groups of different severity were 2.10, 2.68, and 3.62 seconds, respectively. The graphical demonstration of these parameters and the distribution of the values within the groups can be seen in Figure 6. The difference of the new parameter is highly significant between severe (OSDI: 31–100) and mild (OSDI: 0–15) graded subjectively reported symptoms, and significant between moderate (OSDI: 16–30) and severe subjectively graded severity. No significance could be observed between subjects with mild and moderate OSDI values.

The correlation coefficients of the commonly performed procedures and both the uncorrected and the corrected lipid layer stabilization times are shown in Table. The correction factor was greater than 1 in most of the cases, because only six subjects had a larger distance of the eyelid margins than their iris diameter was at the time of the observation.

To check the repeatability of this parameter within each subject, we also determined the variance over the five blinks in each subject (overall mean of the corrected lipid layer stabilization time: 2.80 seconds): the mean value for this intrasubject variance was 0.38 seconds (SD: 0.35 seconds).

Since the mean difference between Subgroup 3 (severe) and Subgroup 1 (mild) was 1.52 seconds, the mean variance of the corrected lipid layer stabilization time was 23% of this subgroup difference. In comparison with the intergroup differences, the variance was 66% of the mean difference between Subgroup 1 and Subgroup 2, and 40% of the mean difference between Subgroup 2 and Subgroup 3. The specificity and the sensitivity at different thresholds were computed by constructing a receiver-operator characteristic curve. Our criteria for DED cases were an OSDI score of 15 or greater and, in addition, one of the following diagnostic characteristics: a Schirmer value of 5 mm or lower, a TFBUT of 5 seconds or lower, or a lissamine green staining score of 4 or greater. A cutoff value of 2.6 seconds results in a specificity of 82% and a sensitivity of 70%.

Discussion

We have developed a novel parameter to extend the diagnostic possibilities for the multifactorial disease keratoconjunctivitis sicca, which we have called the "Corrected Lipid Layer Stabilization Time" (CLST). Due to the high variability of each of the commonly performed procedures, the existing diagnostic parameters typically have to be combined so as to obtain a reliable grading of the severity of the disease. Our proposed objective, noninvasively assessed parameter could provide valuable clinical information on the lipid layer of the tear film, thereby facilitating the accurate classification of this disease into subtypes. The dynamics of the lipid layer seem to be well correlated with the subjectively reported symptoms of the DED, especially in comparison with other parameters. This could be explained by the noninvasiveness and objectiveness of the procedure, which results in a low short-term variability of this parameter.

The variability in the subjectively perceived, as well as in objectively, commonly assessed parameters in dry eye patients makes it hard to determine the best way to classify these patients according to the severity of the disease.[12] To investigate the correlation between the lipid layer dynamics and the perceived severity of DED, we used here a classification based on the OSDI alone. Comparing the mean differences of the CLST values between the different subgroups, the short-term variability can be considered low enough to provide a good repeatability for the classification of severe DED. The separation of mild and moderate forms of DED is less clear (short-term variability is 66% of the mean difference of CLST of Subgroups 1 and 2). As a possible cutoff value for our group of subjects, we chose a CLST of 2.6 seconds. The resulting specificity and sensitivity scores of 82% and 70%, respectively, indicate a good performance of this parameter for the diagnosis of DED, although we would expect

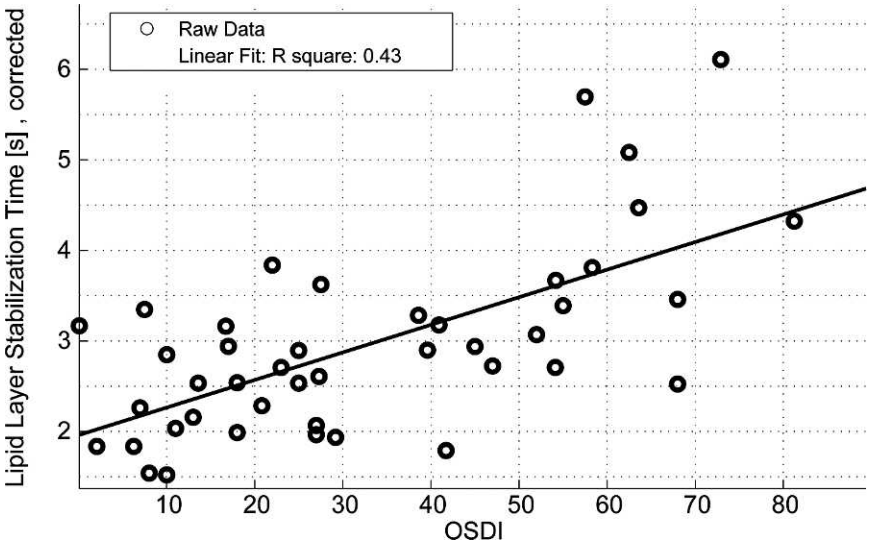

Figure 5. The linear regression model of the corrected lipid layer stabilization time and the subjectively reported severity.

to have better performance when combining the proposed CLST values with other common diagnostic procedures.

The significant correlation of the CLST with the TFBUT indicates the importance of a fast lipid spread for the overall stability of the pre-ocular tear film; a reduced dynamic of this layer is often related to an instable tear film. Our results confirm the data obtained by Goto and Tseng[23] and Yokoi et al.[28] In these studies, it was proposed that the spread of the lipid layer could be related to the amount of tear liquid in the aqueous compartments in the tear film. Our results strengthen this hypothesis by indicating a significant negative correlation with the Schirmer test. This tendency shows that in many cases a high Schirmer value results in a short stabilization time of the lipid layer. This is particularly interesting for choosing the most suitable therapeutic intervention. For example, a high Schirmer value, combined with a high CLST, would indicate a problem with the lipid layer, not with the aqueous layer. In that case, a treatment with lipid-containing tear substitutes could be the preferred solution over a treatment with lubricant eye drops.[29]

The background of the kinetics of the tear film lipid layer spread after a blink action has been reviewed by Bron et al.[3] and Butovich.[2] With the upward movement, the superior tarsal plate draws lipids from the region between the apposed lids. This leads to a rapidly upward propagating lipid layer over the underlying aqueous layer, which finally decays to a stabilization of the tear film lipid layer. It is supposed that initially the polar lipids spread rapidly over the aqueous layer, followed by a retarded movement of the nonpolar lipids.[28] Owens and Phillips[30] described the decay of the lipid spread velocity by a logarithmic function. They investigated the tear film lipid layer

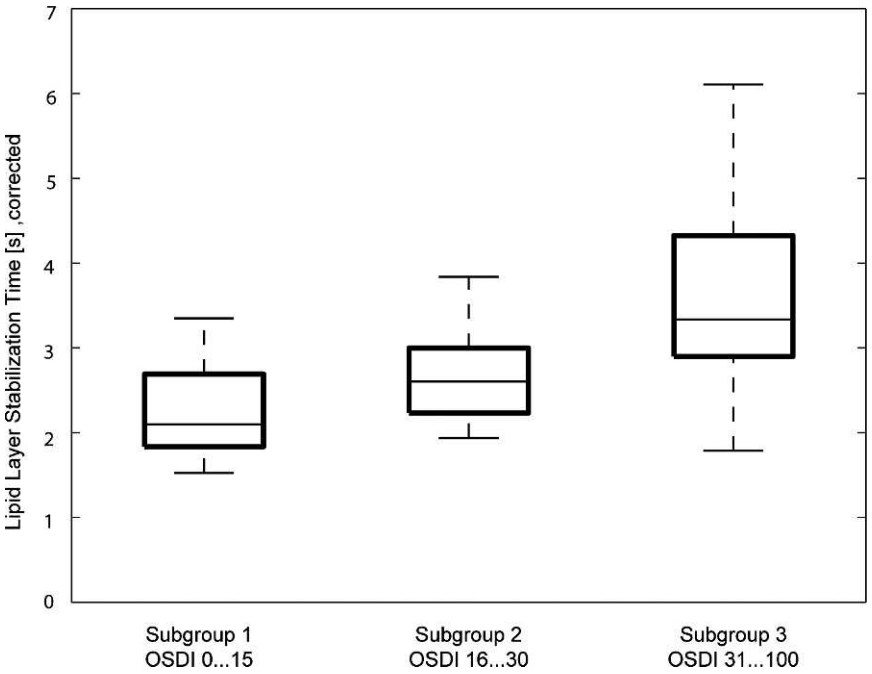

Figure 6. The location and scattering parameters of the different subgroups. *Center-line boxes* indicate the median and 25th and 75th percentiles, and the *error bars* indicate the measurement range.

Table. Correlations of the Uncorrected and the Corrected Lipid Layer Stabilization Time

	Lipid Stabilization Time Uncorrected		Lipid Stabilization Time Corrected	
	Rho	***P***	**Rho**	***P***
TFBUT	−0.303*	0.048	−0.485†	0.001
Schirmer I	−0.291	0.061	−0.431†	0.004
Lissamine green staining	0.223	0.165	0.379*	0.011
Osmolarity	0.074	0.659	0.004	0.861
OSDI	0.371*	0.017	0.498†	0.001

Spearman: *$P \leq 0.05$; †$P \leq 0.01$.

spread through the observation of particle movement in the tear film of healthy adult subjects. The lipid layer movement has been shown to stabilize after approximately 1 second, which is comparable to the uncorrected lipid layer stabilization time acquired by our device within Subgroup 1 (mild). Yokoi et al.[28] used a video interferometer (DR-1; Kowa, Tokyo, Japan) for this purpose, and proposed a linear viscoelastic model as a theoretical attempt to describe the tear film lipid spread.

Even though we tested each of the 59 patients with each of the six tests, our study still has some limitations. For a complete investigation of our newly proposed parameter, a much larger and more heterogeneous patient population should be recruited, to check, for example, its variation with age. Although Bron et al.[3] have reported a good reproducibility of the tear film lipid layer dynamics, some patients have reported a clear change on their subjectively reported severity in the evening compared with the morning. Thus, an extension of our study to investigate the variation of this new parameter with the time of the day, as well as its reproducibility over a number of days would be interesting.

Because of the requirements from the ethics commission, we had to constrain our investigation with the new device in each patient to one eye only. The evaluation and comparison of both eyes could be of interest, as intrasubjective differences between the two eyes have been reported by some subjects. Nevertheless, we believe that this does not bias our results, as the behavior of the pre-ocular tear film acquired with binocularly performed standard procedures was highly correlated between the two eyes in each subject (R^2 TFBUT: 0.9, R^2 Schirmer: 0.8, R^2 osmolarity: 0.8). In six patients it was not possible to reliably acquire the lipid layer stabilization time, however. This was due to the low visibility of the tear film lipid layer pattern in these patients, who typically had prominent bright iris colors. We also observed that the visibility of the tear film lipid layer decreased clearly in patients with meibomian gland dysfuntion and primary Sjogrens syndrome. Although the stabilization time could be obtained reliably in most of these cases, the visibility was not high enough to extract the exact parameters of the kinetics of the tear film lipid layer (e.g., the initial velocity) from the images generated by our setup with the current configuration. For these purposes other devices seem to be more appropriate.[28]

Worth noting are also inherent limitations in the assessment of the subjective symptoms with the OSDI: the OSDI is among other criterions based on the visual performance. Because we included mostly elderly people into the study, a poor score in questions about visual impairments during daily activities (e.g., driving at night) may arise from other ophthalmic diseases, such as age-related macular degeneration or cataract. But because the OSDI has been extensively used by other studies and has been evaluated for its usage in dry eye diagnosis, we believe that this factor does not lead to a biased study selection.[24]

Although a slow lipid spread may be responsible for the hyperevaporation of the tear fluid, the correlation of the CLST and the osmolarity measurement was remarkably low. This could be explained by the fact that only one osmolarity measurement was performed for each eye in contrast to the proposed procedure of Khanal and Thomas.[31] They suggested taking the average of three consecutive measurements to reduce the variability of this test. The sensitivity and specificity of the osmolarity measurement are still under discussion.[32]

Although the sampling rate used in our study was much higher than in comparable previous studies,[22,23] it was still too low to reliably acquire the lid closure dynamics. Details of the lid closure dynamics could be of potential interest to investigate the dynamics of the lipid layer and the dynamics and completeness of eyelid closures in more detail.[33] To acquire a more detailed insight into the interaction between the lipid spread and the underlying aqueous layer, it would be necessary to couple our device with a method that measures the local thickness of the aqueous layer. This could be achieved through optical coherence tomography, for example. Due to the constraints of the different methods, the corresponding experiments would probably have to be conducted sequentially.

As an outlook, the possible connection of a slow lipid layer spread and transient changes in visual performance after a blink could be interesting, since the lipid layer is an important refracting layer for the incoming light. An inhomogeneous distribution could lead to chromatic aberration and image defects. For investigations concerning this question, a coupling of our procedure to methods proposed by Ridder et al.[15] or Benito et al.[14] would be necessary. They both describe different methodologies to assess the transient visual impairment associated with DED.

Our experiments and data analysis showed that for routine usage of the lipid layer dynamics in the diagnosis of DED patients, it would be desirable to have an autofocus system, and an automated data analysis of the acquired videos. Also, we want to make clear that whereas this new parameter provides valuable information about the lipid layer of the tear film, other tests are required for a complete assessment of a DED patient. Thus, the CLST should not be taken as the only parameter to assess the severity of the DED. Rather, we believe that this parameter should be seen as one step to acquire a comprehensive view on the complex construct of the tear film to grade the overall severity of the disease. By providing a direct measurement of the behavior of the lipid layer, the CLST can be valuable for the development of diagnostic and therapeutic patterns for different subtypes. In contrast to the subjective grading of the tear film integrity, our novel device provides an objective, noninvasively assessed parameter for the performance of the lipid layer.

We have also shown the importance of the lid opening on the behavior of the lipid layer. We think that this is important because there was a great variability within patients and it is supposed that a larger lid margin distance leads to a thinner tear film. It is possible that patients with a small quantity of tear fluid compensate for this by reducing the opening of the eye.

Our results underline the importance of the tear film lipid layer and potential of interferometric methods for the diagnosis of tear film performance, in particular if the performance of the lipid layer is corrected for the influence of different eyelid opening behaviors.

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References

1. Tseng SC, Tsubota K. Important concepts for treating ocular surface and tear disorders. *Am J Ophthalmol*. 1997;124:825–835.
2. Butovich IA. Lipidomics of human Meibomian gland secretions: chemistry, biophysics, and physiological role of Meibomian lipids. *Prog Lipid Res*. 2011;50:278–301.
3. Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res*. 2004;78:347–360.
4. Begley CG, Caffery B, Nichols KK, Chalmers R. Responses of contact lens wearers to a dry eye survey. *Optom Vis Sci*. 2000; 77:40–46.
5. Doughty MJ, Blades KA, Ibrahim N. Assessment of the number of eye symptoms and the impact of some confounding variables for office staff in non-air-conditioned buildings. *Ophthalmic Physiol Opt*. 2002;22:143–155.
6. Nakamura S, Kinoshita S, Yokoi N, et al. Lacrimal hypofunction as a new mechanism of dry eye in visual display terminal users. *PLoS One*. 2010;5:e11119.
7. Uchino M, Schaumberg DA, Dogru M, et al. Prevalence of dry eye disease among Japanese visual display terminal users. *Ophthalmology*. 2008;115:1982–1988.
8. Schaumberg DA, Buring JE, Sullivan DA, et al. Hormone replacement therapy and dry eye syndrome. *JAMA*. 2001;286: 2114–2119.
9. Toda I, Asano-Kato N, Komai-Hori Y, et al. Dry eye after laser in situ keratomileusis. *Am J Ophthalmol*. 2001;132:1–7.
10. Nakamura H, Kawakami A, Eguchi K. Mechanisms of autoantibody production and the relationship between autoantibodies and the clinical manifestations in Sjögren's syndrome. *Transl Res*. 2006;148:281–288.
11. Lemp MA. Ocular surface disease. *Arch Ophthalmol*. 1984; 102:194.
12. Subcommittee of the International Dry Eye Work Shop. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology. *Ocul Surf*. 2007;5:108–152.
13. Begley CG, Chalmers RL, Abetz L, et al. The relationship between habitual patient-reported symptoms and clinical signs among patients with dry eye of varying severity. *Invest Ophthalmol Vis Sci*. 2003;44:4753–4761.
14. Benito A, Pèrez GM, Mirabert S, et al. Objective optical assessment of tear-film quality dynamics in normal and mildly symptomatic dry eyes. *J Cataract Refract Surg*. 2011;37: 1481–1487.
15. Ridder WH, Tomlinson A, Huang JF, et al. Impaired visual performance in patients with dry eye. *Ocul Surf*. 2011;9:42–55.
16. Mengher LS, Bron AJ, Tonge SR, et al. A non-invasive instrument for clinical assessment of the pre-corneal tear film stability. *Curr Eye Res*. 1985;4:1–7.
17. Guillon M, Styles E, Guillon JP, et al. Preocular tear film characteristics of nonwearers and soft contact lens wearers. *Optom Vis Sci*. 1997;74:273–279.
18. Mainstone JC, Bruce AS, Golding TR. Tear meniscus measurement in the diagnosis of dry eye. *Curr Eye Res*. 1996;15:653–661.
19. Yokoi N, Bron AJ, Tiffany JM, et al. Relationship between tear volume and tear meniscus curvature. *Arch Ophthalmol*. 2004; 122:1265–1269.
20. Foulks GN. The correlation between the tear film lipid layer and dry eye disease. *Surv Ophthalmol*. 2007;52:369–374.
21. Bron AJ, Tiffany JM, Gouveia SM, et al. Functional aspects of the tear film lipid layer. *Exp Eye Res*. 2004;78:347–360.
22. Goto E, Tseng SCG. Differentiation of lipid tear deficiency dry eye by kinetic analysis of tear interference images. *Arch Ophthalmol*. 2003;121:173–180.
23. Goto E, Tseng SCG. Kinetic analysis of tear interference images in aqueous tear deficiency dry eye before and after punctal occlusion. *Invest Ophthalmol Vis Sci*. 2003;44:1897–1905.
24. Schiffman RM, Christianson MD, Jacobsen G, et al. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol*. 2000;118:615–621.
25. Guillon JP. Non-invasive Tearscope Plus routine for contact lens fitting. *Cont Lens Anterior Eye*. 1998;21:31–40.
26. Tomlinson A, Khanal S, Ramaesh K, et al. Tear film osmolarity: determination of a referent for dry eye diagnosis. *Invest Ophthalmol Vis Sci*. 2006;47:4309–4315.
27. Van Bijsterveld OP. Diagnostic tests in the Sicca syndrome. *Arch Ophthalmol*. 1969;82:10–14.
28. Yokoi N, Yamada H, Mizukusa Y, et al. Rheology of tear film lipid layer spread in normal and aqueous tear-deficient dry eyes. *Invest Ophthalmol Vis Sci*. 2008;49:5319–5324.
29. Craig JP, Purslow C, Murphy PJ, et al. Effect of a liposomal spray on the pre-ocular tear film. *Cont Lens Anterior Eye*. 2010;33:83–87.
30. Owens H, Phillips J. Spreading of the tears after a blink: velocity and stabilization time in healthy eyes. *Cornea*. 2001; 20:484–487.
31. Khanal S, Thomas JM. Barriers to clinical uptake of tear osmolarity measurements. *Br J Ophthalmol*. 2012;96:341–344.
32. Messmer EM, Bulgen M, Kampik A. Hyperosmolarity of the tear film in dry eye syndrome. *Dev Ophthalmol*. 2010;45:129–138.
33. McMonnies CW. Incomplete blinking: exposure keratopathy, lid wiper epitheliopathy, dry eye, refractive surgery, and dry contact lenses. *Cont Lens Anterior Eye*. 2007;30:37–51.