Relationship between corneal sensation, blinking and tear film quality

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Abstract

Purpose: To examine the possible role of corneal sensitivity and tear film quality in triggering a blink by investigating the relationship between blink rate, central corneal sensitivity threshold (CST), ocular surface temperature (OST), tear meniscus height (TMH), tear film quality (non-invasive tear break-up time: NIBUT), and tear film lipid pattern, under normal conditions.

Methods: Forty-two volunteers (average age: 27.76±5.36 years; 11 males) with good ocular health (OSDI <15.0) were recruited for this cross-sectional cohort study. Blink rate, CST (non-contact corneal aesthesiometer, NCCA), minimum and maximum OST in the central and inferior cornea between blinks (thermal infrared camera), tear meniscus height (TMH), and NIBUT and lipid pattern of the tear film (Keeler Tearscope Plus) were recorded on the right eye only.

Results: Median blink rate was 11 blinks/min (IR: 6.95-17.05), CST was 0.35 mbars (interquartile range, IR: 0.30-0.40), minimum OST in the central cornea was 35.15°C (IR: 34.58-35.50) and NIBUT was 34.55sec (IR: 12.45-53.80). Moderate but statistically significant correlations were observed between CST and NIBUT ($r=0.535$; $p<0.001$), CST and blink rate ($r=-0.398$; $p<0.001$), lipid pattern and OST ($r=0.556$; $p<0.001$) and between CST and OST ($r=0.371$; $p=0.008$). The correlations between blink rate and NIBUT ($r=-0.696$, $p<0.001$) and between OST and NIBUT ($r=0.639$; $p<0.001$; Spearman's test) achieved higher significance; this was highlighted by the linear regression model, where NIBUT, minimum central and inferior OST were identified as significant predictor variables.

Conclusions: There is strong evidence for significant interactions between corneal sensitivity, NIBUT, OST and blink frequency emphasizing that ocular surface conditions represent a possible important trigger for the initiation of a blink. However, the mechanisms involved in the initiation of a blink are complex, with local ocular sensory input as only one trigger, along with other external influences and internal factors under cortical control.

Key words: corneal sensitivity, blink, tear film, ocular surface temperature

The human blink mechanism protects the ocular surface against external noxious stimuli and allows the even spreading of the tears over the surface of the eye. Blinking plays an important role in the maintenance of ocular surface integrity, by contributing to the maintenance of ocular surface moisture, drainage of tears, secretion of lipids from the meibomian glands, and the spreading of tear lipids across the pre-corneal tear film.[1-4]

For a blink to occur, the antagonistic muscles of the levator palpebrae superioris and the orbicularis oculi alternately contract in a push-pull fashion.[5] There are three types of blinks: spontaneous endogenous, reflex (both involuntary) and voluntary.[6] Reflex and spontaneous blinks represent a response to different trigeminal, visual and acoustic stimuli, and spontaneous blinks occur unconsciously, without any evident stimulus.[6] A normal blink rate is considered to be 10-16 blinks per minute.[7]

The normal blinking process is greatly variable in blink rate and under cortical control.[6] It is strongly influenced by external factors, psychological and physiological influences, and activity-related factors.[6] Blink rate increases during anxiety,[8] visual fatigue,[9] sleep deprivation,[10] driving,[11] flying,[12] and tasks that require speech.[13] It is reduced during reading or when concentrating on a text on video display:[14] the more difficult the task, the greater the resulting blink inhibition will be. Conversation, anger and excitement markedly increase the blink rate. Furthermore, neurological and psychiatric diseases have an influence, as Cruz et al. reviewed:[15] blink rate is recognised as a clinical marker of central dopaminergic activity; a low blink rate could be recorded in conditions with hypodopamine activity (e.g. Parkinson disease, progressive supranuclear palsy and attention deficit/hyperactivity disorder). Blink rate was found to be high in conditions with hyperdominergic activity (e.g. Huntington disease, schizophrenia, or focal dystonia and neuro-developmental conditions). The afferent pathway of the involuntary blink reflex - elicited by sight or touch – originates in the retina or superficial cornea, respectively, and runs along either the long or short ciliary nerves, respectively, via the ciliary ganglion, along the nasociliary branch of the ophthalmic division of the trigeminal nerve, to the pons and then the medulla oblongata before finally reaching the caudal spinal trigeminal nucleus.[16] From there, impulses are relayed via the medullary pathway, ascending bilaterally to reach the facial nuclei in the pons. These trigeminofacial connections are thought to pass through the lateral tegmental field (medial to the spinal trigeminal nucleus).[16] The efferent arc is conveyed by the facial nerve to the levator palpebrae superioris and the orbicularis oculi.[17]

Despite the cortical control, ocular surface conditions have been associated with blink rate. Blink rate may be influenced by tear film quality,[18,19] since blink rate has been shown to be higher in dry eye patients than the normal population[19] and can be influenced in these patients by use of artificial tears and protective eyeglasses.[20] Nakamori et al. recorded a blink rate in patients with dry eye disease of 34.1±2.4 per min as supposed to normals with to 20.1±1.6 per min.[19] Also, the maximum time during which a person can keep their eyes open was shown to be decreased among dry eye patients.[19] Ocular surface damage has been shown to increase the blink rate.[21]

Hence, it has been hypothesized that corneal sensitivity must play an important role in triggering involuntary blinks, since blink rate reduces when corneal sensation is blocked with a local anaesthetic.[19,22,23] The corneal sensory nerves are integrated into the afferent pathway of the involuntary blink reflex through small unmyelinated (C) and myelinated (Aδ) fibers. C fibers respond to thermal and / or chemical stimuli and many of them have been found to be polymodal and hence, respond to near-noxious mechanical energy as well.[24] Aδ fibers run parallel and deeper (within the basal cell layer) below the corneal surface and are proposed to respond to mechanical stimuli and may also be polymodal nociceptors.[24] The afferent pathway for the ocular surface mediated blink response runs along the short ciliary nerves as described above. The efferent pathway is conveyed by the facial nerve to the lacrimal gland, ensuring basal tear secretion. With a stimulus above a certain threshold (by touch or a sufficient rate of tear evaporation), the levator palpebrae superioris and the orbicularis oculi are also activated, and the lacrimal gland's stronger response leads to lacrimation. Because the reflexes arising from the cornea run through the medulla oblongata before connecting with the ipsilateral and contralateral facial nucleus, it has been postulated that the corneal reflex and the blink reflex use similar trigeminofacial connections.[17]

It has been proposed that involuntary spontaneous blinking is determined by a local corneal reflex that is dependent on corneal sensitivity, possibly triggered by ocular surface cooling when the tear film progressively evaporates.[25] Yap was able to show that blink rate increased when tear break-up time reduced.[18] Mori et al suggested that evaporation-mediated cooling,[26] which occurs during the process of tear break-up, may be detected by thermo-sensitive corneal nerves and thereby provide the signal for a blink to reform the tear film. Several studies have been unable to establish a clear relationship between blink rate, tear break-up time and corneal sensation: Doughty et al. measured invasive tear break-up time (TBUT) using fluorescein and recorded corneal sensitivity with a Cochet-Bonnet aesthesiometer and could not find any correlation

between central corneal tactile threshold and blink rate (r=0.236; p=0.315).[27] However, the Cochet-Bonnet aesthesiometer measures only mechanical corneal sensitivity, as it applies a tactile stimulus to the cornea using a nylon thread. It is not, therefore, the instrument of choice for the measurement of the excitability of thermo-sensitive C fibres in the cornea. In contrast, Ntola, using the non-contact corneal aesthesiometer (NCCA),[28] which uses a fine jet of cooling air as a stimulus and which is designed to measure the activity of corneal C fibres, observed a weak, but not statistically significant, relationship between corneal sensitivity threshold (CST) and inter-blink interval (IBI; r=0.236; p=0.315).[29] Collins et al. showed that infrequent blinking can result from diminished corneal sensitivity; however, they only established a moderate trend for a correlation between CST and blink rate, without statistical significance (r=0.56, p>0.10).[22]

The aim of this study was to examine the possible role of corneal sensitivity and tear film quality in triggering a blink by investigating the relationship between blink rate, central corneal sensitivity threshold (CST), ocular surface temperature (OST), and tear film quality (non-invasive tear break-up time (NIBUT), tear film lipid pattern and tear meniscus height (TMH)) under normal conditions.

Methods

A cross-sectional clinical cohort study method was used. Ethical approval was obtained from the Cardiff University School of Optometry and Vision Sciences Research Audit Ethics Committee, and the study followed the tenets of the Declaration of Helsinki.

Forty-two volunteers were recruited from the staff and patient pool of the Optometry Department, University of Applied Science in Olten (CH). They were invited either by email or by personal invitation in the clinic. All subjects invited to take part in the study were given a subject information sheet explaining the study prior to giving signed consent. The age range was limited to between 20 and 39 years, since corneal sensitivity,[30] OST,[31] and tear film stability[32] have been found to decrease with age. Exclusion criteria for participation in this study were: history of previous ocular surgery including refractive surgery, eyelid tattooing, eyelid surgery or corneal surgery; previous ocular trauma; Sjögren's Syndrome (absence of dry mouth), rheumatoid arthritis, diabetes or ocular infections; current or previous condition known to affect the ocular surface and/or tear film; a score ≥ 15.0 on the Ocular Surface Disease Index (OSDI) questionnaire;[33] medication or use of eye drops known to affect the ocular surface and/or tear

film; pregnancy (on self-report); contact lens (CL) wear one day prior or on the day of this study, as this may affect ocular surface sensitivity.[34-36]

All measurements were made on the right eye at least 4 hours after awakening; between 12:00 and 6:30pm to avoid any possible diurnal bias in corneal sensitivity[37] or tear film stability.[38] Humidity levels and room temperature were controlled to maintain normal office environmental limits (by means of air conditioning), as these variables have been shown to influence OST,[1-4,39] showing a typical increase of 0.15 to 0.2°C per 1°C increase in room temperature.[40]

All subjects completed an OSDI questionnaire and the following measurements were carried out: measurement of blink rate /inter-blink interval (IBI), CST, OST, NIBUT, tear meniscus height (TMH) and tear film lipid pattern grading. In order to avoid an impact of measurements on subsequent ones, there was a break period of at least three minutes between each measurement type.

Ocular Surface Disease questionnaire (OSDI)

The OSDI questionnaire (12-item scale), developed to grade the severity of dry eye disease (DES), is currently used worldwide to discriminate between people with varying levels of ocular surface disease.[33] and is accepted by the U.S. Food and Drug Administration (FDA) for use in clinical trials.[33] For this study, the OSDI questionnaire was applied, in order to fulfil the inclusion criterion that participants were without DES, showing an OSDI score of < 15.0. The official score used to assess dry eye symptoms was: OSDI = (sum of scores) x 25 / (number of questions answered).

Blink rate / Inter-blink interval (IBI)

The blinking pattern of the subjects was recorded using a digital video camera (Sony DCR-TRV27E Digital Handycam, Sony) while viewing a short (7:55 minutes) German documentary ('Brillen für Afrika' from 'Sachgeschichten' from the broadcast 'Sendung mit der Maus' in 2012). The latter was presented on a computer screen (Mac Book Pro, 13 inch with retina display, Apple Computer Inc, Cupertino, CA, USA) at a distance of 2m for a natural viewing situation. The film was chosen to have a 'neutral' theme in order to not generate any emotions capable of affecting the subject's blink rate frequency. All subjects watched the video with best corrected visual acuity. For ethical requirements, the subjects were informed of the video-recording prior to commencement of the study and they signed a separate consent form for this purpose. Since the

blink rate may have been affected by an awareness of being filmed (psychological status may affect blink rate[6]), only the last 5 minutes of film viewing were considered for analysis. This allowed some time for the subjects to 'forget' about the fact that they were being filmed. This 5 minutes duration has been considered ideal for blink behavior analysis by Doughty.[7] The digital recording of each subject's blink frequency was then downloaded to a computer (Mac Book Pro, 3 GHz Intel Core i7 Processor, Apple Computer Inc, Cupertino, CA, USA) and watched again, for analysis with the VLC media player (Version 2.0.5 Twoflower (Intel 64bit), the VideoLAN Team). Blink frequency was analysed in two ways: 1) Blink rate – the average number of blinks per minute; and, 2) Inter-blink interval (IBI) – the average time between blinks in seconds.

Corneal sensitivity threshold (CST)

Corneal sensitivity threshold (CST) was assessed within the central cornea, using the NCCA. This instrument stimulates the ocular surface in a well-controlled, repeatable and consistent manner and has been described previously.[28] Briefly, it stimulates the sensory corneal nerves using a controlled pulse of air at room temperature (and hence considerably cooler than the ocular surface of 34-35°C), aimed at the cornea, which produces a localized area of cooling on the anterior corneal surface. [41,42] The nerves respond to this stimulus and, if the temperature change produced is above threshold, the subject experiences a sensation of cooling. No corneal deformation could be observed during stimulus presentation in one study, hence the authors hypothesized that the air gas stimulus does not have a relevant mechanical element.[41] Using this instrument, the central corneal sensation threshold was measured using a forced-choice, double-staircase technique.[28] Alignment with the cornea was made using a customized slit-lamp attachment that allows accurate positioning of the air-jet at 1 cm away from the central corneal surface. Stimulus duration was set at 1 sec and the time interval between each stimulus presentation was 15 sec.[28] In order to ensure a complete and stable tear film over the cornea, the subjects were asked to make a full, but unforced blink, following which (within 1-2 sec) the stimulus was presented.[43]

Ocular Surface Temperature (OST)

Real-time measurements of OST were carried out on all subjects, with each measurement lasting for the duration of five consecutive natural blinks, using a self-calibrating thermal infrared camera (FLIR A310; thermal resolution 0.08°C, temporal resolution 30 Hz; spatial resolution 320x240 pixel, corneal emissivity 0.95). The camera was placed directly in front of the subject's right eye, at a distance of 25cm and OST was noted in the very centre (at the same location where

the CS threshold is being measured) and at the inferior cornea (2 mm inside the limbus). Grey-scale thermal images were analysed using a purpose-designed computer programme (ThermaCAM Researcher Pro Version 2.9, FLIR Systems, 2006). A mean value for central and inferior OST was recorded 2 sec after each of the 5 consecutive natural blinks and a mean minimum value was determined immediately before each blink (at the time point of maximum evaporation from the ocular surface), as well as the mean temperature difference occurring on the ocular surface between each blink (= OST 2 sec after a blink – min. OST immediately before a blink).

Non-invasive break-up time (NIBUT), tear lipid pattern and tear meniscus height (TMH)

NIBUT and the tear lipid pattern were observed using a Tearscope Plus (Keeler Ltd., Windsor, UK), equipped with a diffuse, cold, light-source. For NIBUT measurement a fine grid was used for better accuracy by enabling earlier observation of small deformation in the grid reflection. Subjects were instructed to blink spontaneously, as they would normally, and then to refrain from blinking after a spontaneous blink. The grid pattern projected onto the cornea was then observed. NIBUT was recorded as the time from the blink until the first distortion in the grid pattern, or the patient expresses a need to blink. Three consecutive measurements were taken and a median value was calculated. The subject was asked to blink gently between measurements to promote re-stabilization of the tear film, for a minimum period of 30sec.

The tear lipid pattern was evaluated according to the classification of Guillon:[44] open meshwork (grade 1), closed meshwork (grade 2), flow / wave pattern (grade 3), amorphous (grade 4), normal colour fringes (grade 5), abnormal colour fringes (grade 6) and globular appearance (grade 7). The pattern was observed after 2 to 3 spontaneous blinks, each time after tear film movement, following a spontaneous blink. No grid was employed for this evaluation.

Tear film stability has been shown to be most stable with the flow, amorphous and normal colour lipid patterns, whereas open meshwork and the abnormal colour fringes have poorer quality: a four-fold increase in tear evaporation could be shown when the lipid layer was absent or when abnormally coloured fringes were observed.[45] According to Guillon, the amorphous lipid layer indicates a stable lipid layer, whereas open and closed meshwork, flow and normal colours describe an average lipid layer, and the globular and abnormal colour fringes describe an unstable tear film.[44] The reduced tear film stability with a thicker lipid layer may be explained by a reduced aqueous tear volume underlying the lipid layer.[46] In order to better reflect tear film

stability, the lipid patterns were divided into the following four grades, with grade 1 representing poor quality and grade 4 representing the most stable tear film quality, which allows for a decrease in tear film stability observed with lipid layers thicker than 'amorphous':[47] Grade 1 – open and closed meshwork, abnormal colour fringes, globular appearance; Grade 2 – wave / flow; Grade 3 – normal colours; Grade 4 – amorphous.

In addition, TMH was measured using Tearscope illumination and an eyepiece graticule on the slit-lamp.

Statistical Analysis

An a priori power calculation was carried out with correlation=0.40, α=0.05, β=0.80 (power=0.80) and correlation ρ H_0 =0, and a sample size of n=37 was obtained (G*Power 3.1). For consideration of possible dropouts, 42 subjects were recruited.

The data did not follow a normal distribution (Shapiro Wilk Test, SPSS Version 20), hence the non-parametric Spearman's test was applied to test the correlations between the relevant parameters (SPSS Version 20). In addition, a robust linear regression analysis for determination of significant predictor variables and their interactions was applied, because the assumptions of collinearity and homoscedasticity were violated with the standard linear regression model (R-statistics, Version 3.1.0). IBI was chosen to be the dependent variable.

Results

Forty-two volunteers participated in this study, of whom 11 were male. Average age was 27.76±5.36 years. The median / interquartile ranges (IR) and mean (±SD) values for all measurements carried are summarized in Table 1. The average ambient temperature in the testing room was 24.7±0.8°C and average ambient humidity was measured to be 41.6±4.5%.

On average, the central OST decreased by 0.15±0.09°C during the period when the eye was open between each blink. The minimum inferior OST was found to be slightly higher than central OST. The inferior OST difference occurring between blinks was slightly higher than in the central part of cornea.

Inter-relationships

The correlations between blink rate / IBI, OSDI score, CST, tear film characteristics and OST are summarized in Table 2, and significant ones are displayed in scatterplots (Figures 1-5).

Moderate but statistically significant correlations between blink rate / IBI and CST (r=-0.398; p<0.001 / r=0.360; p=0.010; Figure 1), NIBUT and CST (r=0.535; p<0.001; Figure 2), lipid pattern and OST (r=0.556; p<0.001) as well as CST and OST (r=0.371; p=0.008) were observed. Stronger correlations were noted between NIBUT and blink rate / IBI (r=-0.696, p<0.001; Figure 3 / r=0.672; p<0.001), tear film stability and blink rate / IBI (r=-0.571; p<0.001; Figure 4 / r=0.519; p<0.001), as well as NIBUT and OST (r=0.639; p<0.001, and between tear film stability and NIBUT (r=0.744; p<0.001; Figure 5).

Robust linear regression analysis

A robust linear regression analysis was applied (R-statistics, Version 3.1.0). This kind of linear regression analysis down weighs data points not fulfilling model assumptions such as normal distribution of random errors. IBI was chosen to be the dependent variable and NIBUT, lipid pattern, tear film stability, CST, minimum central and inferior OST, difference between minimum central and inferior OST (OST gradient) and their interactions represented predictor variables. The following variables and their interactions were found to be non-significant and were hence removed: lipid pattern, tear film stability and CST. Consequently, the final model was carried out only for NIBUT, OST gradient and the interactions between NIBUT and OST gradient as predictor variables. It was statistically significant (p<0.001) and explained 66.3% of the variance ($R^2=0.688$, adjusted $R^2=0.663$).

The model was described as the following:

Dependent Variable = Intercept + A $*IV_1$ + B $* IV_2$ + C$*IV_1 * IV_2$; IV=independent variable.

IBI = 2.600 + 0.05177 * NIBUT – -0.74716 * OST gradient - 0.09407 * (NIBUT * OST gradient).

Power Calculation

A post-hoc power calculation was carried out for the correlations between the parameters CST, NIBUT, blink rate, IBI, tear film stability and min. OST in the central cornea, and the results are summarised in Table 3 (G*Power 3.1): the powers between these parameters ranged between 0.69 and 1.0.

Table 4 summarizes the NIBUT measurements for different lipid patterns and distributions, compared to the Tearscope Plus manual.

Discussion

This study explored the relationship between blink frequency (blink rate / IBI), corneal sensitivity, tear film quality (NIBUT, lipid pattern, TMH and OST) and dry eye symptoms (OSDI) in normal subjects. A non-invasive measurement of tear film break-up was chosen (NIBUT), the habitual blink rate was recorded, and corneal sensitivity was measured with a non-invasive method (NCCA air gas aesthesiometer).

To the authors' best knowledge, this is the first study to show a moderate and statistically significant correlation between blink rate and corneal sensitivity. A stronger correlation between blink rate, NIBUT and OST could be confirmed in this study and was highlighted by the robust linear regression analysis, where NIBUT, OST gradient and their interactions were identified as significant predictor variables for the outcome variable inter blink interval. This supports the hypothesis that the thinning of the tear film before break-up may contribute to triggering a spontaneous involuntary eye-blink.

However, no statistically significant correlation could be found between blink rate and TMH.

The median score for the OSDI questionnaire obtained in this study represented a normal range for a normal population.[33] The median blink rate found in this study was very similar to the average blink rate reported by Doughty in his review paper.[7] The median minimum central OST occurring between blinks was within a normal range for OST of 32.9-36.0°C,[40] and showed very little variability between the healthy participants. The NIBUT measurements obtained in this study on normal subjects were found to be higher than in some other published clinical studies.[45,48,49] This may be explained by the inclusion criterion of a low OSDI value for participation in this study and the young age group. In an age group <45 years, Maissa and Guillon obtained a mean NIBUT of 20.0±13.9s;[49] Thirty-nine percent of the participants in this group study had NIBUT values of >20s. The prevalence of different lipid patterns observed in the current study compared fairly well with those estimated in the Tearscope Plus manual (Table 4).[50]

Isreb et al. observed a positive correlation between lipid layer thickness and tear break-up time (with the use of fluorescein) on 44 eyes with dry eye disease symptoms (r=0.653, p<0.01),[51] confirming that a thicker lipid layer is desirable and is correlated with an optimal tear film stability and tear film characteristics. This may suggest a better tear film stability and hence a longer break-up time with a tear film showing normal colour fringes, rather than showing an

amorphous lipid pattern. In the current study, however, those eyes showing an amorphous lipid pattern exhibited, on average, a longer NIBUT and were therefore graded as having a superior tear film stability (Table 4). According to the Tearscope Plus instruction manual, a lipid layer was graded as 'amorphous', when it appeared as 'thick, white, even and well-mixed that may have shown colours during the blink'.[50] In other words, there may have already been some colours present during the blink that were not visible after the blink. In the current study, this type of lipid layer was found to be more stable (i.e. showing a longer NIBUT rate) than a tear film with a further increase in lipid layer thickness, which continued to exhibit a normal colour pattern after the blink. This finding is in accordance with the results published by Craig et al.[47]

The NIBUT measurements in this study exceeded the median IBI of 4.57 seconds by far – even the lower value of the interquartile range of 12.45 seconds for NIBUT was found to be comfortably higher than the higher value of the interquartile range of 7.04 for IBI. It can hence be concluded that NIBUT exceeded IBI for the subjects participating in this study, as it would be required for normal eyes without dry eye disease, as defined by the ocular surface protection index.[52] Median TMH was found to be normal, as well as the median value for grading of the lipid pattern, which corresponds to a wave pattern and is most commonly found in a normal population. Yokoi et al. could show a good correlation between TMH and the initial velocity of the tear film lipid layer spread after a blink:[53] a shorter spreading time of the tear film after a blink indicates a more stable tear film, and a longer spreading time is characteristic of aqueous tear-deficient dry eye.

Topical anaesthesia has been shown to reduce, but not to abolish, blink rate.[19,22,23,54] A moderate, and statistically significant, correlation between blink rate and corneal sensitivity was shown for the first time in this study.

As mentioned before, no previous study could establish a correlation between blink rate and corneal sensitivity: Ntola observed a correlation of r=0.236 without statistical significance (p=0.315), but had excluded subjects with a TBUT <8 seconds.[29] Collins et al. could show that infrequent blinking can result from diminished corneal sensitivity, however they could only establish a moderate correlation without statistical significance (r=0.56, p>0.10).[22] They recorded blink rate while the subjects were involved in conversation, which may have artificially increased blink rate. Also, their sample group comprised only nine subjects. Doughty et al. did not observe any correlation between corneal tactile threshold (Cochet Bonnet aesthesiometer) and blink rate,

whereby they recorded blink rate for 5 mins. in silence, without any visual stimulation for the participating subjects.[27] However, for the conjunctival tactile threshold, they found a moderate inverse relationship: the lower the conjunctival sensitivity, the higher blink rate was found to be (r=0.588, p<0.001). These results are questionable, as the Cochet Bonnet aesthesiometer has been shown to have many limitations, most importantly a truncated stimulus range and imprecise stimulus application.[28] It may therefore not be sensitive enough for subtle sensitivity differences in normal subjects. Furthermore, a blink rate without any visual stimulation at all may not necessarily be natural. Other research also showed a strong relationship between corneal sensation and tear film drying dynamics.[55]

This study found a statistically significant correlation between IBI and NIBUT and a statistically significant negative correlation between blink rate and NIBUT. Several investigations also found significant negative correlations between blink rate and TBUT, however to variable degrees, as many of these previous studies were affected by the use of fluorescein to measure TBUT or by poor control over the blink recording conditions:[18,56,57] Yap et al. found a strong and statistically significant correlation of r=-0.69 (p<0.01), whereby the patients were filmed with a hidden camera during a period of 5 minutes whilst waiting in the exam room, but relied upon fluorescein TBUT.[18] Al-Abdulmunem also observed a strong correlation in 159 healthy students when they recorded their blink rate by observation in a lecture theatre (r=-0.74, p<0.05), and again used TBUT.[56] Collins et al. established a moderate correlation between TBUT and blink rate, however without statistical significance (r=-0.38, p>0.10). Prause and Norn found a weak to moderate correlation between TBUT and blink rate (r=-0.33; p<0.05) for normals and a stronger, moderate effect for patients with Sjögrens syndrome (r=-0.58; p<0.05).[57] However, they measured blink rate during reading, which may have had an inhibitory effect. Tsubota and Nakamori observed an influence of ocular surface area on blink rate,[58] however these results were disputed by Zaman et al., who could not find any correlation between ocular surface area and spontaneous eyeblink activity in elderly Caucasians.[59] The ocular surface area was unfortunately not measured for the subjects participating in this study.

Acosta et al., as well as Nakamori et al., were able to decrease blink rate during computer work with the use of artificial tears,[19,60] which supports the hypothesis that sensory input from the corneal and conjunctival sensory fibers modulates the neural circuits involved in spontaneous blinking. They concluded that blink rate, at rest, may be partially influenced by extrinsic factors, such as ocular surface conditions, whereas a low blink rate induced by the performance of an

attentive task may be mainly governed by intrinsic neural mechanisms. The resulting strong inhibition of neural blinking mechanisms during the computer task is stronger than the sensory input from the cornea and conjunctiva. It has been suggested that spontaneous blinking originates in the central nervous system and is modulated by internal factors such as fine motor controls, speech centers, emotional and psychological states, cognition and attention.[23] Thus, both central and neural control and local ocular sensory input may jointly act to stimulate blinks.

Each blink spreads warm tears over the ocular surface, after which an immediate heat transfer from the tear film to the environment takes place, leading to a decrease in OST over time after each blink.[61] The tear film destabilizes after a blink, most probably due to evaporation, leading to a cooling response due to the positive latent heat of vaporization as the liquid changes into gas and heat is transferred to the atmosphere.[47] In dry eye disease, the rate of evaporation has been shown to increase, due to a poor lipid layer quantity or quality.[62] Several studies have shown OST to be increased in eyes with a poor tear film quality immediately after the blink, compared to controls.[63] The resulting larger difference in temperature between the eye and the atmosphere may further accelerate the subsequent cooling rate.[64] In the current study, central OST was measured to be slightly lower than in the inferior cornea (in proximity to the inferior limbus), which may be explained by the fact that the vascularized limbal area has been shown to be warmer than the avascular corneal center.[61] The rate of cooling between blinks was similar for the central and inferior cornea in this study. The minimum central and inferior OST temperatures (immediately before a blink) were highly correlated with NIBUT in this study, suggesting a lower cooling rate with better NIBUT measurements. The correlations between OST and lipid pattern/tear film stability were consequently good as well. Also, higher OST measurements correlated well with longer inter-blink intervals, supporting the hypothesis that evaporation contributes to the initiation of a blink. The direct correlation between OST and CST, however, was not observed to be strong in this study.

A statistically significant correlation between corneal sensitivity and NIBUT was found in this study. Situ et al. established a weak to moderate correlation between corneal sensitivity and NIBUT in patients with dry eyes (r=0.31 for cornea, r=0.40 for conjunctiva; air gas aesthesiometry).[65]

The weaker correlation between blink rate and CS, than between blink rate and NIBUT in this study may be due to the feedback loop between NIBUT and the initiation of a blink in healthy

eyes that hinders the activation of superficial corneal nerves. The high median value for NIBUT obtained for the subjects in this study indicated a very good tear film quality. A stronger correlation between CS and blink rate would be expected in eyes during the beginning stages of the dry eye disease process, where a sensitization of the superficial corneal nerves and an increased blink rate have been reported.[66]

All measurements were carried out by only one examiner, therefore masking of the CST, TMH, NIBUT and lipid layer measurements to the examiner was not possible - this is a potential limitation of this study. However, the analysis of blink rate was carried out at a later time point after completion of all data collection on all subjects, in order to avoid a direct subjective comparison between the results.

Conclusion

The mechanisms involved in the initiation of an eye-blink are complex. This study suggests that local ocular sensory input represents one possible trigger for the initiation of a blink, next to other external influences and internal factors that are under cortical control. The stronger correlation between blink rate, NIBUT and OST found in this study, emphasizes the fact that ocular surface condition plays an important role in blink rate.

For future research, it would be interesting to explore if a more significant correlation between ocular surface sensation and blink rate can be established in eyes during the early stages of the dry eye disease process, where the sensitization of the superficial corneal nerves and an increased blink rate have been reported.[66,67]

Acknowledgements

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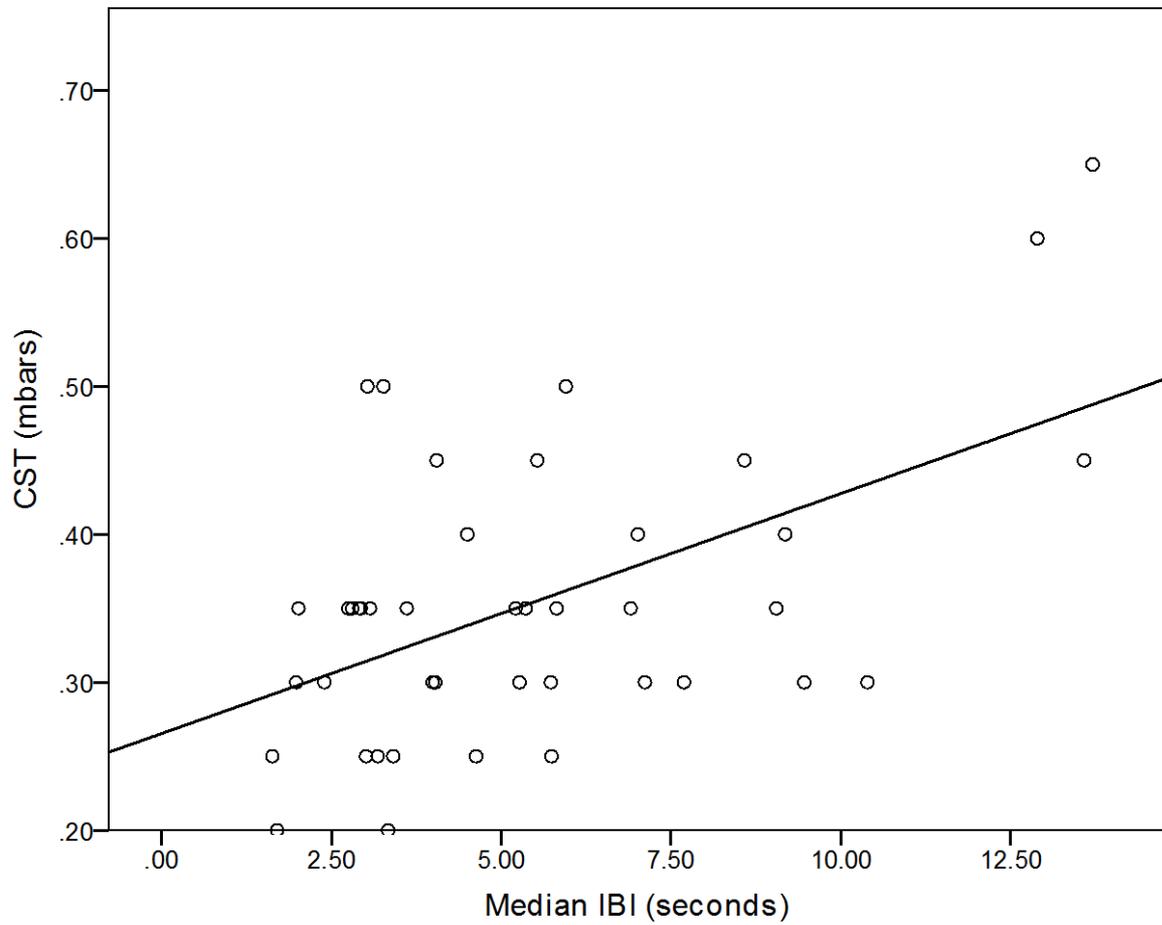
Figures and Tables

Figure 1: Scatterplot for median inter-blink interval (IBI, in seconds) and corneal sensitivity threshold (CST, in mbars); line fitted to median values.
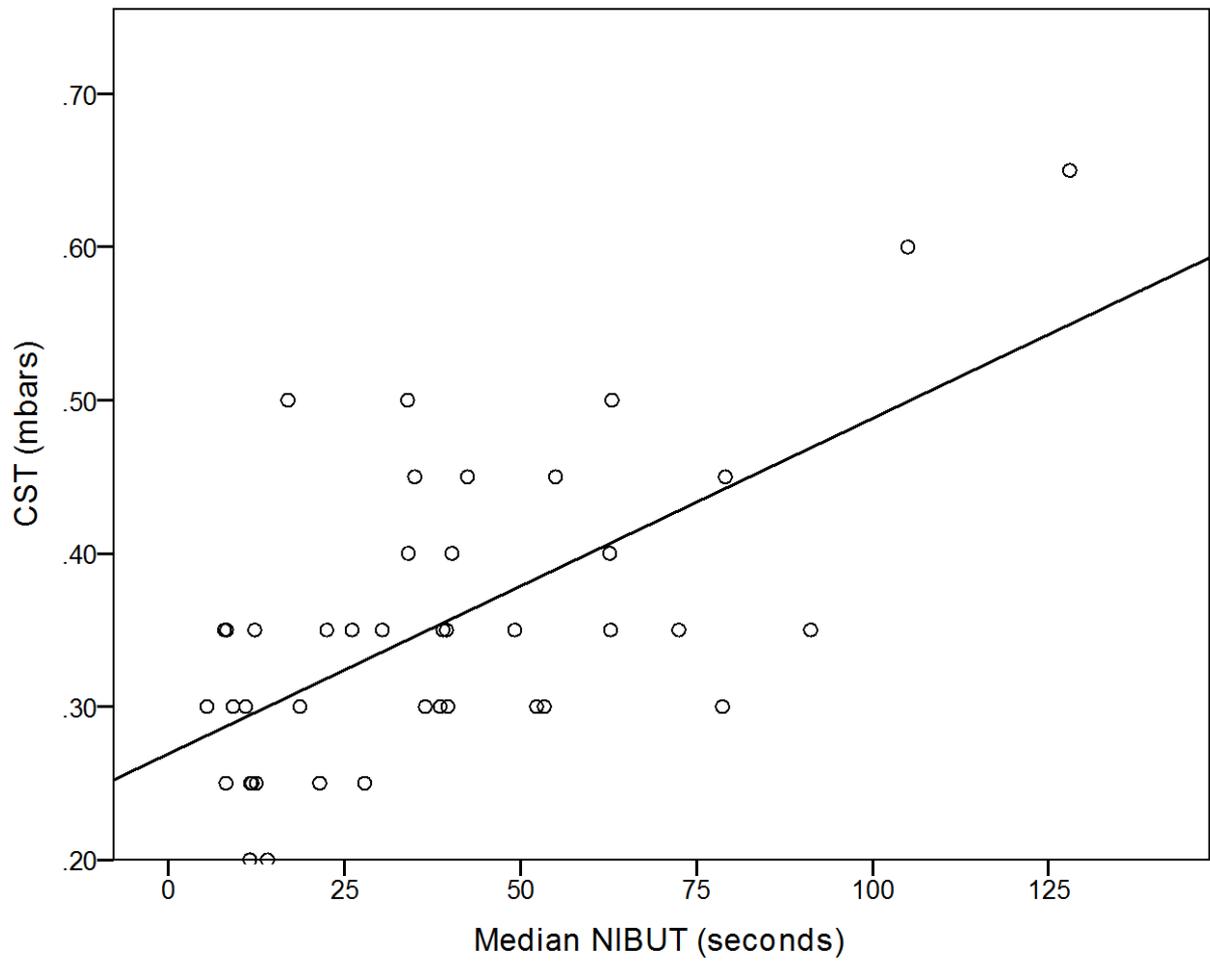
Figure 2: Scatterplot for median non-invasive tear break-up time (NIBUT, in seconds) and corneal sensitivity threshold (CST, in mbars); line fitted to median values.
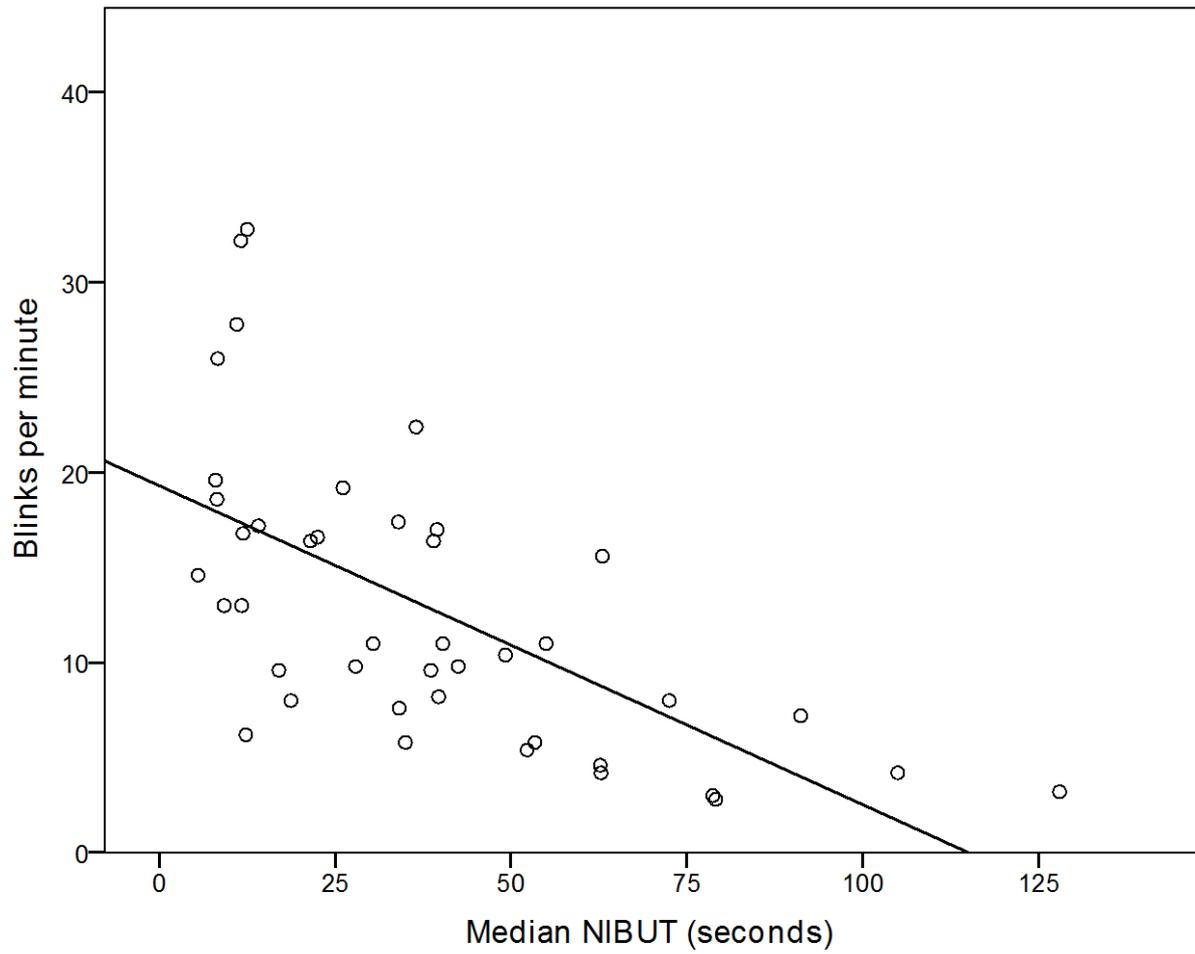
Figure 3: Scatterplot for median non-invasive tear break-up time (NIBUT, in seconds) and blinks per minute; line fitted to median values.
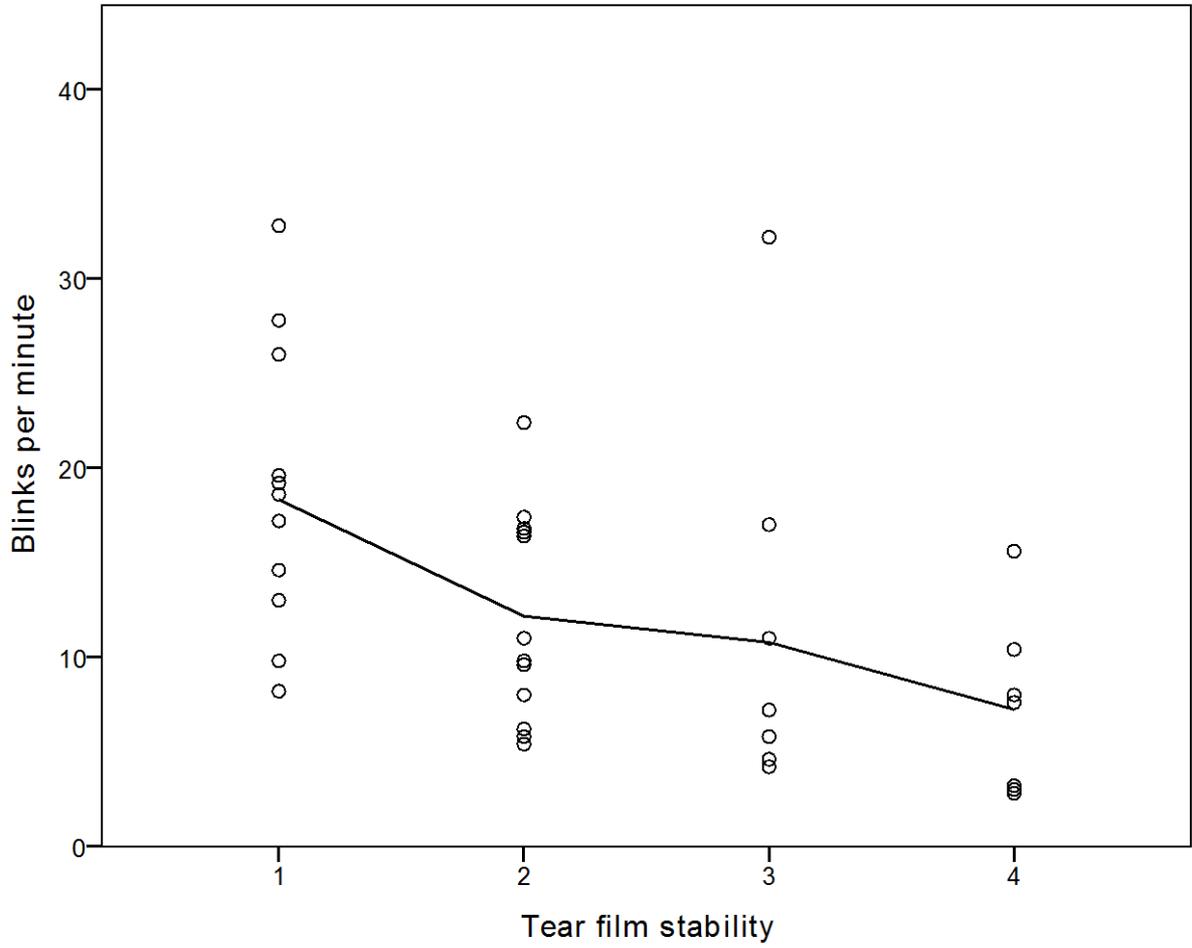
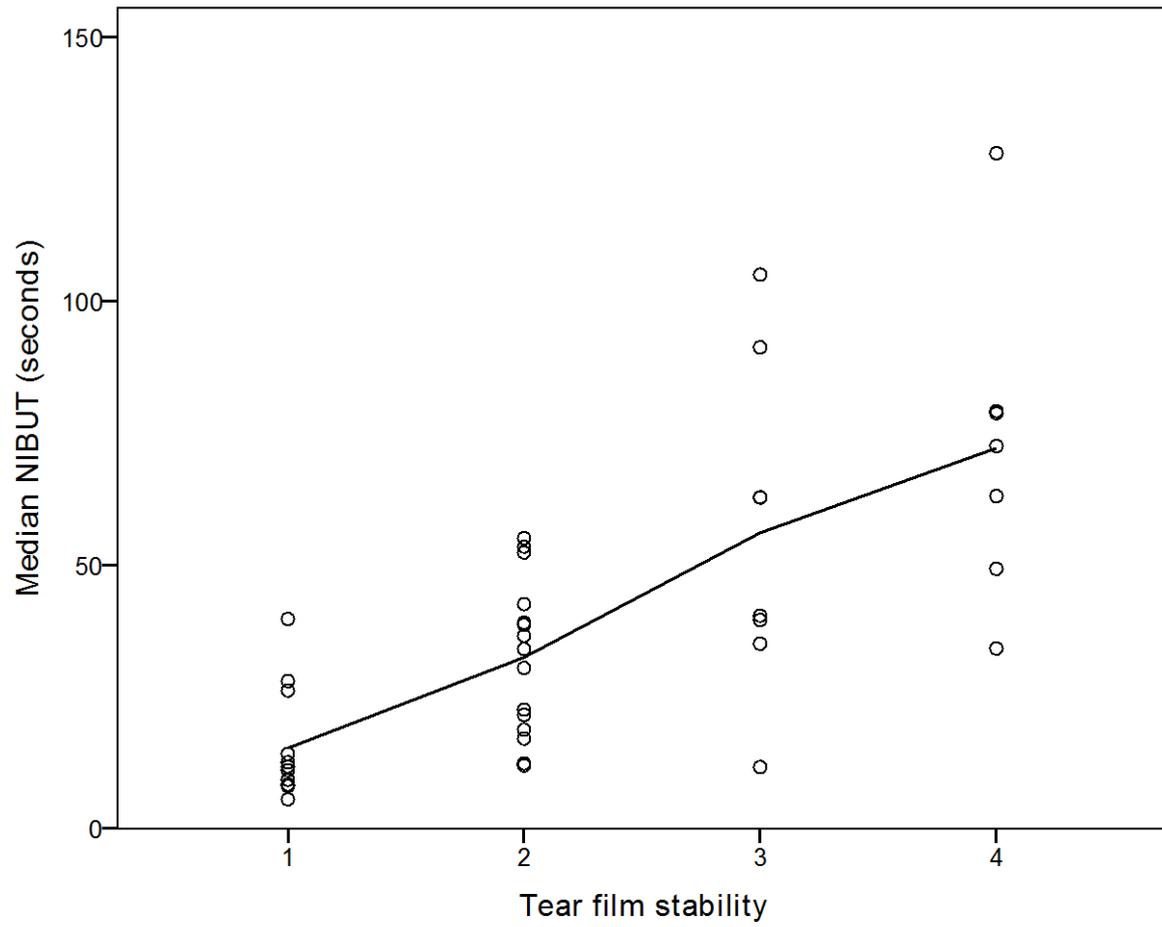**Figure 4: Scatterplot for tear film stability and blinks per minute; line fitted to median values.**

Figure 5: Scatterplot for tear film stability and median non-invasive tear break-up time (NIBUT); line fitted to median values.

	OSDI	Blinks per minute	IBI (secs)	CST (mbars)	Min OST central cornea (°C)	OST difference central cornea (°C)	Min. OST inferior cornea (°C)	OST difference inferior cornea (°C)
Median / IR	8.30 / 4.20-14.60	11.00 / 6.95-17.05	4.57 / 3.03-7.04	0.35 / 0.30-0.40	35.15 / 34.58-35.50	0.15 / 0.10-0.20	35.30 / 34.78-35.60	0.20 / 0.10-0.20
Mean±SD	8.86±4.92	12.83±7.64	5.44±3.18	0.35±0.98	34.98±0.68	0.15±0.09	35.11±0.74	0.16±0.09

	NIBUT (secs)	Lipid pattern	Tear film stability	TMH (mm)
Median / IR	34.55 / 12.45-53.80	3.0 / 2.75-4.25	2.00 / 1.0-3.0	0.23 / 0.20-0.30
Mean±SD	38.58±28.62	3.29±1.44	2.24±1.05	0.23±0.75

Table 1: Median / Interquartile Range (IR) and Mean (±Standard Deviation) of the following measurements: OSDI (ocular surface disease index), blinks per min., inter blink interval (IBI), corneal sensitivity threshold (CST), min. ocular surface temperature (OST) central cornea, OST central cornea difference between blinks, min. OST inferior cornea, OST inferior cornea difference between blinks, non-invasive tear break-up (NIBUT), lipid pattern, tear film stability and tear meniscus height (TMH).

	OSDI	Blinks per minute	IBI	CST	Min. OST central cornea	OST difference central cornea	Min. OST inferior cornea	OST difference inferior cornea	NIBUT	Lipid pattern	Tear film stability	TMH
OSDI	--											
Blinks per minute	0.122 (p=0.220)	--										
IBI	-0.112 (p=0.239)	-0.964 (p<0.001)	--									
CST	-0.306 (p=0.024)	-0.398 (p<0.001)	0.360 (p=0.010)	--								
Min. OST central cornea	-0.039 (p=0.466)	-0.478 (p=0.001)	0.474 (p=0.001)	0.371 (p=0.008)	--							
OST difference central cornea	-0.238 (p=0.064)	-0.217 (p=0.084)	0.181 (p=0.126)	0.252 (p=0.053)	0.298 (p=0.028)	--						
Min. OST inferior cornea	0.015 (p=0.406)	-0.545 (p<0.001)	0.552 (p<0.001)	0.330 (p=0.017)	0.937 (p<0.001)	0.249 (p=0.122)	--					
OST difference inferior cornea	-0.039 (p=0.112)	-0.204 (p=0.097)	0.167 (p=0.145)	0.192 (p=0.112)	0.427 (p=0.012)	0.414 (p=0.003)	0.426 (p=0.002)	--				
NIBUT	-0.194 (p=0.110)	-0.696 (p<0.001)	0.672 (p<0.001)	0.535 (p<0.001)	0.639 (p<0.001)	0.362 (p=0.009)	0.620 (p<0.001)	0.392 (p=0.005)	--			
Lipid pattern	-0.049 (p=0.380)	-0.561 (p<0.001)	-0.562 (p<0.001)	0.325 (p=0.018)	0.556 (p<0.001)	0.289 (p=0.032)	0.500 (p=0.001)	0.381 (p=0.006)	0.675 (p<0.001)	--		
Tear film stability	-0.291 (p=0.031)	-0.571 (p<0.001)	0.519 (p<0.001)	0.534 (p<0.001)	0.518 (p<0.001)	0.390 (p=0.005)	0.488 (p=0.001)	0.333 (p=0.015)	0.744 (p<0.001)	0.697 (p<0.001)	--	
TMH	0.022 (p=0.444)	-0.163 (p=0.429)	0.25 (p=0.216)	0.049 (p=0.380)	0.116 (p=0.232)	0.144 (p=0.182)	0.069 (p=0.286)	0.064 (p=0.344)	0.028 (p=0.429)	0.354 (p=0.011)	-0.040 (p=0.401)	--

Table 2: Correlation coefficients (r) between dry eye symptoms (OSDI), blinks per minute / inter-blink interval (IBI), corneal sensitivity threshold (CST), minimum ocular surface temperature (OST) in the central cornea, OST difference between blinks in the central cornea, minimum OST in the inferior cornea, OST difference in the inferior cornea, non-invasive tear break-up time (NIBUT), lipid pattern, tear film stability and tear meniscus height (TMH), (Spearman's test, SPSS Version 20).

	Blinks per minute	IBI	CST	Min. OST central cornea	NIBUT	Tear film stability
Blinks per minute	--					
IBI	1.00	--				
CST	0.77	0.69	--			
Min. OST central cornea	0.95	0.95	0.70	--		
NIBUT	1.00	1.00	0.98	1.00	--	
Tear film stability	0.99	0.98	0.98	0.98	1.00	--

Table 3: Post-hoc power calculation (G*Power 3.1).

Lipid pattern observed	Mean NIBUT ± standard deviation (seconds)	Distribution in current study (%)	Distribution published by the Tearscope Plus manual (%)
open meshwork	11.50±6.82	16.7	15
closed meshwork	11.37±2.91	7.1	14
wave / flow	32.37±14.69	35.7	29
amorphous	72.09±29.63	16.7	19
normal colour	56.01±30.85	21.4	17
abnormal colour	33.8±8.34	4.8	not stated
globular	-	-	6

Table 4: NIBUT measurements for different lipid patterns and distributions, compared to the Tearscope Plus manual.