

Article

Ocular Surface Temperature in DED under Natural Non-Controlled Blinking Conditions

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Abstract: Infrared (IR) thermography is a tool to non-invasively assess the tear film temperature. The aim was to analyze ocular surface temperature (OST) variations in dry eye disease (DED) and control eyes under natural non-controlled blinking conditions. Imaging was performed with a thermal camera (*FLIR Systems Inc.*) at 30 Hz framerate in 79 participants (39 DED (62.5% women, average age 48 ± 20 years) and 40 control (46.2 % women, average age 38 ± 13 years)) using non-contact IR thermography camera. Data acquisitions were performed in natural blinking conditions for 40 s. IR images were analyzed using a custom algorithm that calculates the OST indexes: mean OST, OST at the start and at the end, minimum and maximum OST, and tear evaporation rate (TER). No significant differences were found between groups in any thermal parameter analyzed (paired comparisons *t*-test, $p > 0.05$). In conclusion, the findings of this study did not reveal significant differences between DED and control eyes under natural non-controlled blinking conditions. However, the presence of clinical signs in the control group may affect the results, highlighting the role of DED diagnosis criteria.

Keywords: dry eye disease; thermography; ocular surface temperature; tear evaporation rate; natural blinking



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1. Introduction

Dry eye disease (DED) has been identified as a multifactorial condition of the ocular surface commonly encountered in the ophthalmic practice [1,2]. The prevalence of DED has been estimated to be between 5 and 50% [3], highlighting the outstanding impact that this disorder has on the quality of life [4,5]. Currently, DED diagnosis and monitoring are still challenging for clinicians [6] due to its multifactorial nature. According to the Tear Film and Ocular Surface Society's Dry Eye Workshop (TFOS DEWS II, 2017), DED diagnostic protocol should include both symptomatology questionnaires, as a screening tool, and homeostasis markers tests [7]. However, most of the clinical tests used for DED diagnosis have limitations as they are semi-invasive, subjective, and/or have insufficient sensitivity and specificity and low repeatability [8]. In fact, there is a lack of agreement between clinical signs and the severity of DED symptoms, making DED diagnosis difficult [9,10]. Thus, efforts are made to develop more objective, less invasive, and more repeatable methods and technologies to obtain a more reliable DED diagnosis.

Thermographic imaging has been recently applied in different disciplines of biomedicine [11–13] as a potential diagnostic and preventive device to evaluate stress reactions. Infrared (IR) thermography has been used to detect changes in surface temperature across a range of anatomical regions, the eye being where this method fits better [14,15]. The ocular surface (OST) evaluation through IR thermography is gaining importance as a potential non-invasive method to assess the ocular surface [11,16]. OST has shown to depend on

several extrinsic and intrinsic factors, such as ambient and body temperature, contact time with the vascular palpebral conjunctiva (i.e., lid closure), ocular inflammation, age, and blinking [11,17,18].

In this context, DED is well-known to be related to ocular surface inflammation [19], and its prevalence increases with age [3], both key factors influencing OST [17,18]. Therefore, it is to be expected that OST will experience changes in DED eyes compared to healthy eyes [20].

At this point, the question arises whether thermography can be a potential tool for DED diagnosis. Therefore, it would be expected that eyes with DED had faster TER and, accordingly, cooler OST. However, there are discrepancies between studies in this regard [21–23]. However, most studies evaluating the association between OST and DED perform the measurements under controlled blinking conditions.

Knowing that the stability of the tear film decreases with time when the eye is open, and that OST is dependent on the tear film stability [11,24], maintaining the eyes open for a longer period than the normal blink could influence the results. To the best of our knowledge, no previous research has investigated OST under natural non-controlled blinking conditions in DED eyes. Therefore, this study aims to evaluate OST variations under natural non-controlled blinking conditions in DED eyes and healthy controls.

2. Materials and Methods

This was a prospective study conducted at the Faculty of Optics and Optometry at the Complutense University of Madrid. The research protocol was approved by the Ethics Committee of San Carlos University Hospital (Madrid, Spain) (Approval code 16/279-E) and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from each participant prior to the start.

2.1. Subjects

The inclusion criteria for this study were: age ≥ 18 years, ability to complete the questionnaires, and understanding of the study procedures. The exclusion criteria were history of any active ocular disease different from DED or meibomian gland dysfunction (e.g., corneal ulcers, herpes simplex, keratitis, etc.), any uncontrolled severe systemic disease that may affect the eye (e.g., Sjögren's syndrome, diabetes type II, dermatological disease, etc.) or any ocular surgery or trauma that could affect the tear film distribution or produce any eyelid margin abnormality. None of the participants received eye drop installations or wore contact lens within 6 h before measurements.

The classification criteria for DED subjects were based on the presence of symptoms and signs as described by the TFOS diagnosis criteria report [7]. Subjects who showed an Ocular Surface Disease Index (OSDI) score ≥ 13 points and presented at least one sign of altered homeostasis (tear film stability: Non-Invasive Tear Film Break-Up (NIBUT) < 10 s (sec); Tear Film Osmolarity (TFO) ≥ 308 mOsm/L; ocular surface staining > 5 corneal spots) were included in the DED group. Subjects who reported no subjective DED symptoms were included in the control group regardless the presence of signs.

The sample size was calculated based on previous data published by Nosch et al. [25]. The standard deviation was assumed to be 0.68 [25]. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 38 subjects were necessary to recognize as statistically significant a difference greater than or equal to 0.31 units [26]. A drop-out rate of 0% had been anticipated because all tests were performed in the same visit.

2.2. Clinical Assessment

All subjects underwent careful examination to identify exclusion criteria and a specific evaluation of classification variables (TFO, NIBUT, corneal staining, and OSDI) and thermography metrics. For every patient, all tests were performed in the same order (from the least to the most invasive) by the same examiner (LR) with a 5-min interval between tests in a room with monitored temperature and humidity. All measurements were performed only

in the right eye and three consecutive measurements were taken, except for TFO, which was measured once in each eye.

TFO was assessed using the TearLab Osmolarity System (TearLab Corp, San Diego, CA, USA) following manufacturer's instructions. NIBUT was performed using the Keratograph 5M (K5M, Oculus Optikgeräte, Wetzlar, Germany). Corneal integrity was assessed by instilling fluorescein in the eye using a fluorescein strip (Tiedra Laboratories, Madrid, Spain) that was previously wetted with saline solution (Saline solution, AVIZOR, Madrid, Spain). Two minutes after the instillation, the corneal staining was graded using the Oxford scoring scheme (0–5 score). The evaluation was performed using a cobalt blue filter incorporated in the illumination system together with a yellow Wratten #12 filter. Subjects who showed an Oxford score ≥ 1 were considered positive for corneal staining (>5 corneal spots). The OSDI score was calculated as (sum of scores for all questions answered $\times 100$)/(total number of questions answered $\times 4$) [27].

2.3. Thermal Recording

A non-contact IR thermography camera (FLIR A325; FLIR Systems Inc., Wilsonville, OR, USA) was used to measure the OST. This thermal camera has an image resolution of 320×240 pixels, a sensitivity of 50 mK, and an accuracy of $\pm 2\%$. It can detect temperatures that range from $-20\text{ }^{\circ}\text{C}$ to $120\text{ }^{\circ}\text{C}$. Data acquisitions were done with a frame rate of 30 Hz and tear emissivity was set to 0.98 [28]. The camera incorporates a macro-lens to obtain a clear image of the eye and it was aligned with the geometric center of the cornea. The distance between the IR camera and the eye was established according to the best focus point in each subject. Subjects were adapted to the room environmental conditions for at least 20 min [29] before OST evaluation in order to stabilize the eye temperature and minimize the experimental error.

Before OST assessment, body temperature was measured using a digital thermometer (Citizen Digital Thermometer CTA303, Citizen System, Vega Technologies Inc., Taipei, Taiwan) and subjects with body temperature $\geq 37\text{ }^{\circ}\text{C}$ were excluded. During OST measures, subjects were instructed to maintain a stable head position on the chin- and forehead rest, close their eyes for 3 s and afterwards open the eyes and look straight forward for 40 s while blinking naturally.

2.4. Thermal Analysis

The software provided by the manufacturer was used to obtain OST data (ResearchIR software, FLIR Systems Inc., Wilsonville, OR, USA) that was further exported and analyzed using a custom written algorithm developed with Matlab (R2017b v9.3, TheMathWorks Inc., Natick, MA, USA). The region where the analyses were performed (region of interest, ROI) was manually determined by a single examiner and kept constant for the entire recorded sequence (see Figure 1). The calculated OST indices were analyzed over the complete sequence (40 s) and additionally over the last complete interblink interval (IBI).

2.4.1. Complete Thermal Recording

Those frames affected by blinks registered during the complete thermal recording were detected and removed from subsequent analysis. The OST indices [30] calculated along the complete recorded sequence (40 s) include mean OST, OST at the start (OST start) and the end of the sequence (OST end), and tear evaporation rate (TER) calculated as the amount of water loss from the exposed ocular surface according to Tan et al. (measured in W/m^{-2} that refers to power loss per area) [31].

2.4.2. Last Complete Interblink Interval (IBI)

From all recorded IBIs, the last IBI was considered the most realistic and closest to the subjects' natural blinking conditions. The OST indices for the last complete IBI include mean OST (mean OST-IBI), OST at the start (OST-IBI start) and the end of the last IBI (OST-IBI end) and tear evaporation rate (TER-IBI). Identifying the maximum and

minimum values at the start and end of the IBI contributes to gaining an understanding of the thermoregulating effect of blinking. Therefore, the following indexes were included for analysis: the maximum value of OST at the beginning of the IBI (OST-IBI star max), the minimum value of OST at the beginning of the IBI (OST-IBI star min), the maximum value of OST at the end of the IBI (OST-IBI end max), and the minimum value of OST at the end of the IBI (OST-IBI end min). These indices were calculated as the average of the five maximum or minimum values, respectively.

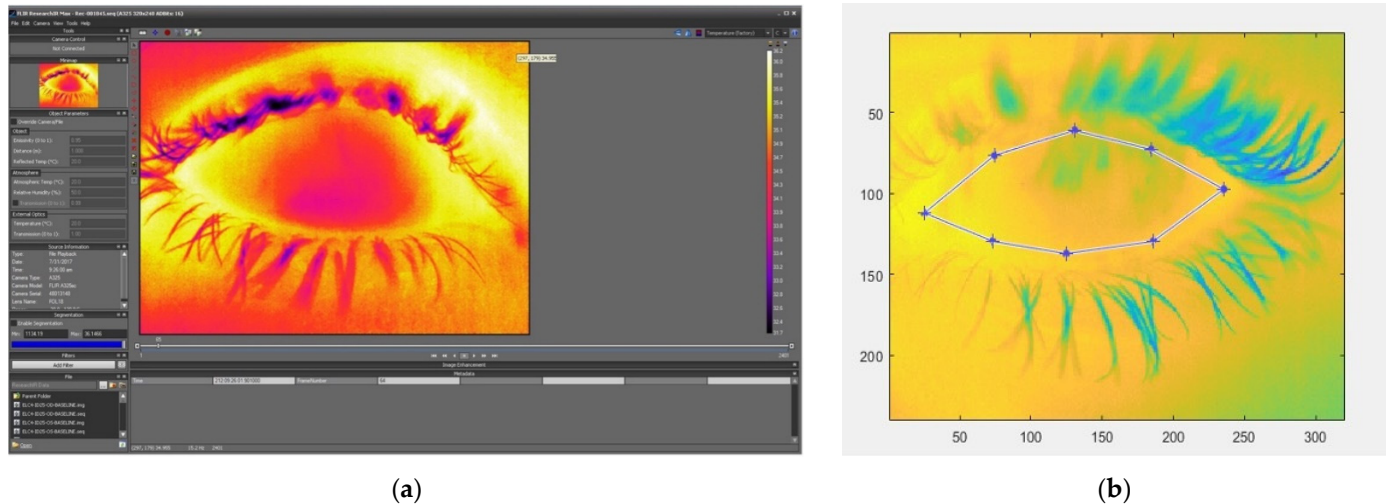


Figure 1. Thermal analysis: (a) Representative screenshot of ResearchIR software used to analyze OST; (b) Example of manual demarcated ROI.

2.5. Statistical Analysis

Statistical analysis was performed using SPSS software (SPSS Version 25; SPSS, Chicago, IL, USA). Normality of the data distribution was tested using the Kolmogorov–Smirnov test. Independent t-test (if normality of the data was assumed) and Mann–Whitney test (if normality of the data was rejected) were used for comparisons between groups. Mean and standard deviation (SD) and median and interquartile range (IQR) were used to describe the group values for the temperature and tear film parameters. A significance level of $p < 0.05$ was set for all statistical tests.

3. Results

A total of 79 eyes of 79 patients were evaluated in the present study; 40 patients were included in the DED group and 39 in the control group. The sex distribution was 37.5% (15) men and 62.5% (25) women in the DED group, and 53.8% (21) men and 46.2% (18) women in the control group. There were no differences between sex. The mean age was 48 ± 20 years (range 19 to 88) in the DED group and 38 ± 13 years (range 22 to 67) in the control group. DED group was significantly older compared to controls ($p = 0.042$).

Table 1 summarizes the outcomes for the complete 40-s recording and in the last complete IBI. The average number of blinks during the sequence was 13 ± 6 and 11 ± 4 for DED and control groups, respectively. There were no differences between groups ($p = 0.069$).

No significant differences between DED and control groups were found for any of the OST or TER indices ($p > 0.05$ all), neither for the complete sequence nor in the last IBI.

Table 1. Thermographic data for the complete 40 s of sequence and for the last complete interblink interval (IBI).

		DED	Control	<i>p</i> -Value
Complete thermal recording (40 s)	OST (°C)	34.8 ± 0.7	35.0 ± 0.6	0.308 ^α
	OST start (°C)	34.9 ± 0.7	35.0 ± 0.6	0.363 ^α
	OST end (°C)	34.8 ± 0.7	35.0 ± 0.6	0.411 ^α
	TER (W/m ^{−2})	109.6 ± 8.8	107.6 ± 7.0	0.286 ^α
Last complete interblink interval	OST-IBI (°C)	34.8 ± 0.7	35.0 ± 0.7	0.400 ^α
	OST-IBI start (°C)	34.9 ± 0.7	34.9 ± 0.7	0.319 ^α
	OST-IBI end (°C)	34.8 ± 0.7	34.8 ± 0.7	0.722 ^α
	TER-IBI (W/m ^{−2})	109.9 ± 8.7	108.2 ± 6.6	0.368 ^α
	OST-IBI start min (°C)	33.5 ± 1.5	33.6 ± 1.4	0.716 ^β
	OST-IBI start max (°C)	36.0 ± 0.6	36.1 ± 0.6	0.314 ^α
	OST-IBI end min (°C)	33.6 ± 1.2	33.5 ± 1.6	0.437 ^α
	OST-IBI end max (°C)	35.9 ± 0.7	36.1 ± 0.6	0.328 ^β

OST: ocular surface temperature; OST start: OST at the start; OST end: OST at the end; TER: tear evaporation rate; OST-IBI: ocular surface temperature in the interblink interval; OST-IBI start: ocular surface temperature in the interblink interval at the start; OST-IBI end: ocular surface temperature in the interblink interval at the end; TER-IBI: tear evaporation rate in the interblink interval (units: W/m^{−2}, power loss per area); OST-IBI start min: the minimum value of OST at the beginning of the IBI; OST-IBI start max: the maximum value of OST at the beginning of the IBI; OST-IBI end min: the minimum value of OST at the end of the IBI; OST-IBI end max: the maximum value of OST at the end of the IBI. ^α T-test (parametric); ^β Mann–Whitney test (non-parametric).

4. Discussion

IR thermography has been used as a tool to non-invasively assess the tear film, both statically and dynamically. Morgan et al. [21] emphasized the potential of this technology for tear film assessment and, later on, Purslow and Wolffsohn demonstrated the existing connection between the tear film stability and OST [24]. The present study contributes to those developments by providing information about the analysis of OST dynamic variations of DED eyes and controls under more realistic and natural non-controlled blinking conditions.

The outcomes of the current study revealed no significant differences between DED and control groups in any of the thermal parameter analyzed. Besides age being considered as a key factor affecting both DED and OST [21,32,33], the OST between groups was not significantly different despite the DED group being significantly older. Additionally, the number of blinks during the 40 s sequence was similar between both groups. DED patients blinked 13 ± 6 times and control patients blinked 11 ± 4 times, which translates into approximately 20 and 17 blinks per minute, respectively in agreement with the normal blinking rate previously reported [34–36].

It is known that when the lids are opened, the ocular surface loses heat by convection, radiation and evaporation [22]. However, there is some controversy regarding OST changes in DED eyes. In the current study, no differences in thermal measurements were observed between DED and control subjects for the recorded sequence. These results are in disagreement with some studies [32,37], which observed that DED subjects presented cooler OST and faster cooling rates compared to controls. Craig et al. found that DED patients have a significantly lower temperature in the geometric center of the cornea than controls [22]. Tan et al. found lower temperatures in DED subjects after 5 and 10 s of eye-opening (geometric center of the cornea, mean OST, minimum and maximum temperature, and different areas of the eye) [38]. Kamao et al. also observed a greater decrease in OST in DED eyes at 10 s after eye-opening and, in addition, they found that eyes with a shorter FBUT are more likely to have a decrease in the OST, but the mean OST in DED eyes did not differ from controls [23].

Conversely, a study conducted by Morgan et al. showed an increased mean OST in DED subjects (32.38 ± 0.69 °C) in comparison to controls (31.94 ± 0.54 °C) [21]. The authors attributed the higher OST in DED patients to inflammation or increased hyperemia [21,39]. Additionally, other research studies reported no significant difference in the mean OST immediately after eye-opening between DED and control subjects [40,41].

Concerning tear evaporation, a wide range of results has been reported [42]. The outcomes of the current study showed no differences in TER between groups. Craig et al. found higher tear film evaporation rate, lower tear film stability and lower OST in DED eyes compared to healthy eyes [22]. Mathers et al. found significantly higher TER in DED patients compared to normal eyes, suggesting that this high rate is caused by a decreased tear production, exacerbating DED [43].

However, it should be noted that the studies previously discussed performed the thermal analysis under controlled blinking conditions, in opposition to the current work, which might explain the differences with our results. As previously stated by Purslow and Wolffsohn, the blink action brings the tear film and the ocular surface into close contact with the vascularized palpebral conjunctiva, maintaining the temperature constant [24]. While the eye is opened, the ocular surface temperature decreases. Hence, maintaining the eyes open longer than the normal blinking could increase the overall TER and decrease the average temperature, exacerbating the differences between DED and healthy subjects. The present study was performed under natural non-controlled blinking conditions to represent the natural state of the eye as accurately as possible.

The IBI is the interval time between two consecutive blinks, and it has been demonstrated that, together with other parameters related to the blink, it is useful for distinguishing between healthy and DED subjects [30,44,45]. Considering that the subjects were asked to close their eye for 3 s right before the start of the recording, the first IBI might be more susceptible to bias. Therefore, the last complete IBI was considered the most realistic and natural interval. In the current study, no differences were found between groups for any thermal parameter characterized in the last IBI. Conversely, Abreau et al. measured the mean OST during a 5-s IBI and found significant differences between DED and controls, but the assessment was performed under controlled blinking conditions [46].

Another factor that could have affected the results of the current study is the DED diagnosis criteria. It is well known that due to the disease's complexity and unclear etiology, diagnostic criteria for DED are still not fully standardized. DED diagnosis according to DEWS II includes symptomatology assessment (via OSDI questionnaire) as a screening tool, and the scheme also allows consideration of various related manifestations, such as non-obvious disease involving ocular surface signs without related symptoms [7]. According to these criteria, subjects with signs but no symptoms were classified as the control group in the current study. Knowing that OST significantly correlates with some clinical DED signs [47], having subjects with signs in the control group could have equalized the mean temperature with the DED group. In fact, 30 of the 39 subjects of the control group presented at least one clinical sign. The mean OST of those 30 subjects was 34.9 ± 0.7 °C, whereas the OST of the other 9 with no signs was 35.4 ± 0.2 °C, showing a 0.5 °C increment. Considering the coefficient of repeatability of OST in 0.31 °C [26], this increment reveals the importance of the DED diagnosis system. Therefore, it seems that the presence of clinical signs cools the temperature. This is supported by a recent study showing positive correlations between OST and TMH and Schirmer's test values, which are often decreased in DED [47]. However, this hypothesis needs to be further studied.

The study presents some limitations. First, only one ROI was established for the entire ocular surface, limiting the information obtained by area (for example, nasal and temporal conjunctiva). On the other hand, it was established in this way to avoid errors delimiting the ROI in the different areas, since in the thermal image it is not possible to observe the anatomical limits of the different structures. Secondly, as mentioned previously, our control group consisted of subjects without symptoms but with clinical signs, which could influence the results. For this reason, this approach should be addressed in future studies.

5. Conclusions

In conclusion, the thermal findings of the present study did not reveal significant differences between control and DED eyes under natural non-controlled blinking conditions. To the authors' best knowledge, this is the first study to show OST differences between DED and control eyes under natural non-controlled blinking conditions. However, the presence of clinical signs in the control group may affect the results highlighting the role of DED diagnosis criteria.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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