Chapter 2

Aqueous Deficient Dry Eyes and
Thermal Fluctuation

2.1 Chapter summary

In this chapter, I have studied the thermal fluctuation patterns occurring at the ocular surface of the left and right eyes for aqueous deficient dry eye (ADDE) patients and control subjects by thermal imaging. The experiment was conducted on 42 patients (84 eyes) with aqueous deficient dry eyes and compared with 36 healthy volunteers (72 eyes) without any history of ocular surface disorder. Schirmer's test, Tear Break-up Time, tear Meniscus height and fluorescein staining tests were conducted. Ocular surface temperature measurement was done, using an FL-IR thermal camera and thermal fluctuation in left and right eyes was calculated and analyzed using MATLAB. The time series containing the sum of squares of the temperature fluctuation in the ocular surface were compared for aqueous deficient dry eye and control subjects. Significant statistical difference between the fluctuation patterns of control and ADDE was observed (p<0.001 at 95% confidence interval). Thermal fluctuations in left and right eyes are significantly correlated in controls but not in ADDE subjects. The
possible origin of such correlation in control and lack of correlation in the ADDE subjects is discussed.

2.2 Introduction

2.2.1 Dry Eye and its Classification

Dry eye is a disorder of the tear film due to tear deficiency or excessive evaporation [35–37], which causes damage and discomfort to the ocular surface. However, recent review by Savini et al. [38] points out that the term “dry eye” includes spectra of alterations of the ocular surface with varying etiology and pathophysiology. While the exact definition of dry eye is debated, the general consensus that was accepted in International Dry Eye workshop is that the dry eye is a part of the ocular surface disease, which includes both Aqueous Deficient Dry Eye (ADDE) and Evaporative Dry Eye (EDE), lid related diseases (such as Meibomian Gland Dysfunction (MGD) and anterior blepharitis), allergic conjunctivitis and other inflammatory, infective or iatrogenic conditions [39]. In most cases, dry eye patients suffer from excessive tear evaporation rate because of the instability of the tear film [40–42]. The cause of ADDE is a lack of aqueous tear secretion by the lacrimal glands, whereas EDE is most often caused by MGD, in which the lipid secretion required to control evaporation and maintain a normal tear film is abnormal.

2.2.2 Conventional Method for Dry Eye Detection

Interestingly, like the problems in the disease definition, the methods to detect and score the dry eye are also non-unique. Staining based techniques, Schirmer’s test (ST) and the staining based tests are mostly semi-invasive in nature. The techniques commonly used in diagnosis are ST, Tear Break-up Time (TBUT) and ocular surface staining, but such tests are imperfect and may lead to incorrect diagnosis [43, 44]. The film stability test (TSAS) on the other hand
is based on complex refractometric measurements [38]. Apart from this the dynamics of the tear film by different biophysical aspects have been studied but they still possess some limitations [45].

2.2.3 Exploring New Methods for Dry Eye Diagnosis

In this chapter an independent approach for identification and scoring of dry eye disease (in this case ADDE) was described. The method is based on Infrared (IR) thermography or IR thermal imaging [46, 47]. The reason behind this is its non-invasive nature as well as its accuracy in measuring a wide range of temperatures on the macro as well as on the micro level. Measurement of the ocular surface temperature (OST) using an IR-based thermal camera known as ocular thermography [48–52], was first introduced by Mapstone [53–56]. Recently, there has been a rapid advancement in infrared thermography and its application in Ophthalmology that has paved the way for measurements of OST. Infrared thermography is a non-contact and non-intrusive, temperature measuring technique, capable of displaying real-time surface temperature distribution [57, 58]. The growing fascination with OST is primarily due to the information gained from it, which may reflect the physical as well as
2.3 Materials and methods

Infrared cameras have earlier been used to analyze normal eyes in different age groups [60] and in addition have been used to detect patients with dry eye syndrome and it has been reported that dry eye patients have a greater decrease in the OST than the normal individuals under normal circumstances [61]. However, in a later study it was observed that there is a smaller decrease in the OST as compared to the normal individuals [62, 63]. This discrepancy in IR thermal imaging has not been studied extensively, although the patterns of thermal images of dry eye patients appeared to be more irregular as compared to the control group [64–66]. Recently, it has been shown that the temperature profile is smoother for normal individual as compared to the dry eye patients [67]. Although, it has been reported earlier that cooling of the ocular surface occurs more predominantly in dry eye patients than in normal individuals. Earlier researchers have reported the absolute temperature difference between dry eyes and normal subjects; also to be noted is the fact that they analyzed the data based on single eye. The expected result that the higher cooling of the ocular surface as a result of lesser tear film stability was what was reported. Apart from the above study various mathematical models and algorithms have been developed in order to correctly localize the corneal surface for accurate measurement of OST in disease states [68–71].

2.3 Materials and methods

In this study ocular surface thermal fluctuation in left ($\Delta T(t)L$) and right ($\Delta T(t)R$) eyes for 42 ADDE patients and was compared with 36 normal individuals without any symptoms of ocular surface disorder. Here, $\Delta T(t)$ refers to the temperature fluctuation of the ocular surface. This study was approved by the institutional ethics committee prior to its commencement. The research adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects.
2.3.1 Subjects

Forty two (84 eyes) ADDE patients (17 male; 25 female: mean age 35.2 ± 4.3 years) were recruited from the outpatients department. Thirty six (72 eyes) control subjects (16 male; 20 female: mean age 28.4 ± 3.1 years) were recruited from the staff of the hospital. Inclusion criteria were devised such that all patients had ADDE symptoms, were not taking any artificial tear supplement, had either a Tear break-up time and fluorescein score of more than 3 out of 15 [72], a Schirmer’s test result of less than 10 mm in 5 minutes, Tear meniscus height of 0.18 mm or less [73] and two or more symptoms of dry eye according to the McMonnies Dry Eye questionnaire [74]. Patients suffering from Meibomian gland dysfunction (MGD) were excluded in this study. Diagnostic criteria for MGD [75] include (a) slit lamp microscopy to detect any abnormality of meibomian gland mass, (b) gland expressibility, (c) quantification of gland dropout, (d) Telangiectasia of meibomian gland orifice. The control subjects were asymptomatic of ADDE, Schirmer’s test of >20mm, tear breakup time of >10 seconds, had no previous history of ocular or general pathology and were not taking any topical or systemic medication that could interfere with tear production.

2.3.2 Testing Condition

Infrared thermal camera (Model no. FLIR SC 305, FLIR SYSTEMS AB, Sweden) was used to monitor the OST for ADDE patients and the control group. The camera has a thermal sensitivity of <0.05°C at + 30°C, spatial, temporal and image resolution of 1.36 mrad, 9 frames per second and 320x240 pixels respectively, with spectral range between 7.5-13µm. Subjects adapted to the prevailing room temperature for at least 10 minutes before OST of the right and left eye were recorded [76]. Room temperature and humidity were monitored and controlled at 22.0 ± 0.5°C and 45 ± 5%. Subjects were restricted from (a) topical application of any eye drop (b) any food or drinks intake within 2 hours prior to experiment (c) and any strenuous physical activity that would affect the ocular surface temperature. $\Delta T(t)$
2.4 Results

was calculated by using MATLAB analysis software, MATLAB version 7.9.0. R2009b, Matworks USA.

2.3.3 OST Measurement Parameters

During the measurement the participants were asked to keep their eyes open for 15 seconds without any blink and in this condition thermal sequence of the ocular surface were obtained. The reading was acquired after 2 seconds of eye opening.

2.3.4 Statistical Test

The temperature fluctuation of the 72 control eyes (36 X2) and 84 ADDE eyes (42X2) were respectively combined to produce two arrays one representing the control and the other ADDE. The unpaired student t test was then performed. Validity of the null hypothesis (implying no difference between the control and the ADDE groups) was then tested at 95% confidence interval (H=0, implying null hypothesis, and H=1 implying significant difference between control and ADDE).

2.4 Results

Figure 2.2 (a) and (b) represents a typical thermogram of an individual and the region of the ocular surface (i.e. the cornea) for which temperature is measured. Figure 2.3 (a) represents $\Delta T(t)$ (left and right eye) of a given control, whereas Figure 2.3 (b) shows the $T(t)$ of the OST for the same control subject. Similarly, Figure 2.4 (a) and (b) depicts the $\Delta T(t)$ and $T(t)$ (left and right eye) for an ADDE patient. The above Figures (2.3 (a), 2.3 (b), 2.4 (a) and 2.4 (b)) describe the variation in temperature of the ocular surface along with the actual OST.

Interesting results were obtained when the time course for all the control (36) and ADDE patients (42) data are plotted against $\Delta T(t)L$ and $\Delta T(t)R$ as shown in Figure 2.5 (a) and (b),
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Fig. 2.2 (a) A typical thermogram image of an individual. (b) Representing the region of ocular surface (in this case the cornea) denoted by a circle for which OST and temperature fluctuation was measured.

From this figure it may be noted that ADDE individuals exhibit an irregular ocular surface thermal variation (which may be either more heating or cooling compared to the control group) in contrary to the earlier report (which only considered cooling, taking about 11 subjects). The range of temperature fluctuation for ADDE patients ($\Delta T(t)L$ and $\Delta T(t)R$) and control is provided in Table 1 ($p<0.001$). Irregular temperature fluctuation rather than monotonic decay was observed for ADDE patients when time course for $T(t)-\mu$ was plotted as shown in Figures (2.5 (c) and (d)).

In Figure 2.6 (a) and (b), the sum squared temperature fluctuation $\Delta T(t)_{tot}$ is plotted against $\Delta T(t)L$ and $\Delta T(t)R$ respectively. The figures clearly indicate that cooling (i.e. $-ve \Delta T$) or to say minimal temperature deviation is more frequently observed in controls whereas a fluctuating rise and fall in (Ocular Surface Temperature) OST is more often seen in the ADDE patients. The range of sum square temperature fluctuation is given in Table 2.1.

Finally in Figure 2.7 (a) and (c), the distribution of temperature fluctuation in $\Delta T(t)L$ and $\Delta T(t)R$. The striking feature in these figures is that while the control shows minimal difference between $\Delta T(t)L$ and $\Delta T(t)R$ there is a notable shift of distribution of the temperature fluctuation of the right and left eye in case of ADDE patients. Higher temperature range is
2.4 Results

(a) The synchronous acquisition of left and right eye (control) temperature fluctuation $\Delta T(t)$ profile respectively plotted against time. $\Delta T(t)$ denotes ocular surface temperature fluctuation.

(b) Left and right eye $T(t)$ profile of ocular surface (control). Here, $T(t)$ denotes ocular surface temperature.

Fig. 2.3 Ocular Surface Temperature and its fluctuation for a Control individual.

(a) The synchronous acquisition of left and right eye (ADDE patients) $\Delta T(t)$ profile plotted against time.

(b) Left and right eye $T(t)$ profile of ocular surface (ADDE patients).

Fig. 2.4 Ocular Surface Temperature and its fluctuation for an ADDE patient.
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(a) Time course plot of $\Delta T(t)_L$ for all ADDE patients and control. Red and Blue dots indicates ADDE patients and control respectively.

(b) Time course plot of $\Delta T(t)_R$ for all ADDE patients and control.

(c) Time course of $T(t)_L - \mu_L$ for 42 ADDE patients and 36 control is represented. $\mu$ represents mean ($T(t)$).

(d) Time course of $T(t)_R - \mu_R$ for 42 ADDE patients and 36 control is represented.

Fig. 2.5 Time course plot for 36 Control and 42 ADDE patients.

Table 2.1 Represents the temperature range of OST for ADDE patients and Control (left and right eye)

<table>
<thead>
<tr>
<th>OST</th>
<th>Temperature Range ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T(t)_L$ (Control)</td>
<td>33.26 - 36.26</td>
</tr>
<tr>
<td>$T(t)_R$ (Control)</td>
<td>33.00 - 36.19</td>
</tr>
<tr>
<td>$T(t)_L$ (ADDE patients)</td>
<td>30.33 - 39.47</td>
</tr>
<tr>
<td>$T(t)_R$ (ADDE patients)</td>
<td>30.96 - 38.54</td>
</tr>
<tr>
<td>$\Delta T(t)_L$ (Control)</td>
<td>-1.96 - 0.45</td>
</tr>
<tr>
<td>$\Delta T(t)_R$ (Control)</td>
<td>-1.39 - 0.46</td>
</tr>
<tr>
<td>$\Delta T(t)_L$ (ADDE patients)</td>
<td>-2.09 - 2.46</td>
</tr>
<tr>
<td>$\Delta T(t)_R$ (ADDE patients)</td>
<td>-1.52 - 1.86</td>
</tr>
<tr>
<td>$\Sigma T(t)_{tot}$ (Control)</td>
<td>0 - 3.60</td>
</tr>
<tr>
<td>$\Sigma T(t)_{rot}$ (ADDE patients)</td>
<td>0 - 8.91</td>
</tr>
<tr>
<td>$T(t)_L - \mu$ (Control)</td>
<td>-1.64 - 1.36</td>
</tr>
<tr>
<td>$T(t)_R - \mu$ (Control)</td>
<td>-1.85 - 1.34</td>
</tr>
<tr>
<td>$T(t)_L - \mu$ (ADDE patients)</td>
<td>-4.54 - 4.59</td>
</tr>
<tr>
<td>$T(t)_R - \mu$ (ADDE patients)</td>
<td>-3.93 - 3.62</td>
</tr>
</tbody>
</table>
2.5 Discussion

Apart from its use in mammography and in other fields of science \[46, 47\] IR thermal imaging has become a reliable and effective tool not only to measure OST, but also to detect any subtle or minor change in temperature occurring on the corneal surface. Previously, various teams have reported their findings regarding the difference in the OST between dry eyes and healthy volunteers, but most of their studies were steady-state based and hardly any inference was drawn on the basis of the temporal OST dynamics. The topographical variations in OST by infrared thermography have been reported \[70\] but not the temporal OST dynamics.

The actual source of thermal radiation originating from the ocular surface detected by IR camera is primarily that of the tears \[77, 78, 50\]. Continuous flow of tears across the

![Fig. 2.6 Different representation of time course plot for Control and ADDE patients.](image)

(a) $\Delta T(t)L$ vs. $\Delta T(t)_{\text{tot}}$ for 42 ADDE patients and 36 control. Red and Blue dots represents ADDE patients and control.

(b) $\Delta T(t)R$ vs. $\Delta T(t)_{\text{tot}}$ for 42 ADDE patients and 36 control.

also present in case of ADDE patients when T(t) distribution of OST is plotted (Figure 2.7 (b) and (d)). See Table 2.1 for range of temperature distribution.

Finally, $\Sigma T(t)_{\text{tot}} = \Sigma T(t)_{L} + (\Delta T(t)\text{R})^{2}$ was evaluated from the time series data for control and ADDE and the two sets were subjected to student t test (as described in the Methods section). $H=1$ was found at very high degree of confidence level ($\sim 95\%$), at 95% confidence level the $p$ being 0.001.
Fig. 2.7 Frequency histogram plot of temperature distribution.
ocular surface is related to the tear film stability [40]. Assessment of the tear film dynamics by different biophysical [45] aspects as well as the thermodynamic study on the effect of tear film on OST have been studied earlier [79] ADDE patients have insufficient tear quantity in comparison to control subjects and therefore show irregular $\Delta T(t)$ as represented in the data (Figure 2.5 (a), (b), (c) and (d)). In this study only ADDE were included patients while excluding EDE and MGD. Increased tear evaporation rate is the feature that characterizes EDE. In ADDE, due to insufficient tear production, increased evaporation arises in response to poor tear film stability [41, 42]. OST is mainly affected by tear film stability [76, 80] which is one of the main reasons for dry eyes syndrome [81, 39]. Ideally, the two groups involved should be age matched because aging is one of the main causes of abnormal tear film and dry eye syndrome, as reported earlier the average OST decreases with age [60]. In order to nullify the effect of age, only age matched subjects were included in this study.

In this investigation I did not adopt the common practice of recording the thermographic sequence, in which the subjects were asked to close their eyes before data acquisition. Instead the thermal sequences were obtained for a 15s period (although 10s sequence does not show significant change in the results), during which the subjects kept their eyes fully open, without blinking (blink suppression). Since prolonged eyelid closure leads to warmer ocular surface, such sequence may produce artifact $\Delta T(t)$ results. Warmer ocular surface is likely to change the fluidity of meibomian lipid in tear film [82] and consequently produces varying $\Delta T(t)$ on the OST. Besides this, the subject’s eyelid were not lifted as done by Tan et al [83], which could lead to induce reflex tearing [54] altering $\Delta T(t)$ of the eyes. In this study I have emphasized on two parameters, one is $\Delta T(t)$ determination instead of $T(t)$ of the ocular surface. Although, even if taken into consideration $T(t)$ of the ocular surface, the results obtained were similar to temperature fluctuation as shown in Figures (2.5 (c) and (d)) and other important criteria is the paired acquisition of $\Delta T(t)$ for both left and right eye (i.e. $\Delta T(t)_{\text{L}}$ and $\Delta T(t)_{\text{R}}$). Any stability related factor should involve time based measurements of
temperature. This temporal aspect is emphasized for the first time in this study. The time based fluctuation study reveals ADDE patients are more susceptible to uncontrolled thermal fluctuations compared to control. Moreover they show both heating and cooling of the ocular surface. This is in sharp contrast with the control where the thermal variations are more or less constant. We have measured the $\Delta T(t)_{L}$ and $\Delta T(t)_{R}$ which are $\Delta OST$ rather than $OST$ in the respective eyes. They show both upward and downward trends implying a competition of tear film evaporation and tear production. It may be noted that it is not the $OST$ increment but its fluctuation that is more irregular in case of ADDE patients, which is indicative of a less stable thermal steady state in ADDE patients.

In other words, the result suggests that thermal fluctuation on the ocular surface in case of ADDE exhibit a much wider range compared to control as depicted by Figure 2.5 (a) and (b). The range of $\Delta T(t)_{L}$, $\Delta T(t)_{R}$, $\Delta T(t)_{\text{tot}}$ for ADDE patients and control is summarized in Table 2.1. It is clear from Table 2.1 that ADDE patients show a higher temperature distribution range in all cases. The reason for calculating the sum squares of the temperature fluctuation is that, in case of stochastic processes sum squares always provides us a robust estimate of the amplitude of fluctuation. In the present context the time course of temperature variation is a completely new discipline for which there is no established method for analysis. The sum square provides us a reliable trend for representations of patients and control groups, despite significant individual variation among each group. Lastly, the two attributes (i.e. $\Delta T(t)_{L}$ and $\Delta T(t)_{R}$ ) that differentiates ADDE patients from control individuals are further illustrated in the histogram plot (Figure 2.7 (a) and (c)). In this figure, it was shown that synchronous thermal fluctuation in $\Delta T(t)_{L}$ and $\Delta T(t)_{R}$ in case of control subjects are highly correlated, such high temperature fluctuation correlation having absent in case of ADDE patients. In case of ADDE the co-occurrence of positive temperature fluctuation in both the eyes are permissible whereas in the control such possibility is non-existent. The reason behind this variation in thermal fluctuation among the two groups may be due to an unstable tear film
of the ADDE patients. The ocular surface of the ADDE is not uniformly spread with tears as in case with control, this leads to higher thermal fluctuation in the former group. Briefly, the source of thermal fluctuations in the ocular surface can be traced from three different routes (a) the reduced thickness of the tear film causes cooling down, (b) differential internal metabolic events near the ocular surface for ADDE patients, (c) presence of differential proteins that amplifies the effects (a) and (b) and causes this fluctuation. It is unlikely that any one of the above factors is the sole cause for the observed irregular thermal variation in ADDE patients.

### 2.6 Highlights

- Synchronous acquisition of ocular surface temperature for left and right eye of an individual.
- Time series analysis of thermal variations occurring on the ocular surface for control and ADDE groups.
- Range of ocular surface thermal fluctuations greatly determine the presence of the diseased state.
- The method can be used as a model system for diagnosis of ADDE patients.
- Time series to network conversion enabled quantification of time dependent thermal imaging data. The network metrics provides a quantification tool for ADDE classification.