ASSESSMENT OF DRY EYES USING OCULAR SURFACE THERMOGRAPHY

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T4 was ± 0.21 for dry eye and ± 0.16 for controls. A typical standard deviation for CT was ± 0.19 for
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List of Abbreviations

\(^{\text{DEWS}}\) - Dry Eye Workshop
\(^{\text{ADDE}}\) - Aqueous-Deficient Dry Eye
\(^{\text{MGD}}\) - Meibomian Gland Dysfunction
\(^{\text{DED}}\) - Dry Eye Disease
\(^{\text{DES}}\) - Dry Eye Syndrome
SDE - Symptomatic Dry Eye
NDE - Non-Dry Eye
OST - Ocular Surface Temperature
ROI - Region of Interest
MOST - Mean OST of the ROI
GCC - Geometrical Centre of the Cornea
T1 – Extreme temporal conjunctiva
T4 – Extreme nasal conjunctiva
CT – Mid-temporal Conjunctiva
LT - Temporal limbus
LN - Nasal limbus
CN – Mid-nasal Conjunctiva
MinT - Minimum temperature of ROI
MaxT - Maximum temperature of ROI
RTD - Radial temperature difference
SD – Temperature standard deviation of the ROI
TVF - Temperature Variation Factor
CV - Compactness Value
TDV - Temperature Difference Value
\(^{\text{FBUT}}\) - Fluorescein Break-Up Time or Test
\(^{\text{NIBUT}}\) - Non-invasive Break-Up Time or Test
TMH - Tear Meniscus Height
CES – Corneal Epithelial Staining
\(^{\text{PRT}}\) - Phenol Red Thread Test
\(^{\text{TFT}}\) - Tear Ferning Test
\(^{\text{DEQ}}\) - Dry Eye Questionnaire
Mscore – McMonnies DEQ score
Scount – symptom count using McMonnies DEQ
\(^{\text{OSDI}}\) - Ocular Surface Disease Index
CCT – Central corneal temperature
TFLL – tear film lipid layer
ROC – Receiver operating characteristics
AUC – area under the ROC curve
Y – Youden’s index
DP – Discrimination power
A – Asymptote
S – Scale
GR – Growth rate

Abstract

Assessment and diagnosis of dry eye disease (DED) is a challenging task. The conventional ways of diagnosing DED are problematic due to their invasiveness, poor test reliability and significant test duration. Previously, ocular surface thermography has been shown to be able to detect early inflammation and dry eye. However, its diagnostic ability and ocular temperature metrics that can best diagnose DED are not clear.

The objectives of this thesis were manyfold. First, the prevalence of dry eye in Singapore population was investigated as a helpful basis for the rest of the project. A cross-sectional dry eye survey was carried out using McMonnies dry eye questionnaire. Members of the public were interviewed at 46 (out of 62) selected mass rapid transit stations in Singapore and its vicinity. 1004 questionnaires were collected from participants aged 15 - 83 years and various ethnicity. Prevalence of symptomatic dry eye (SDE) was found to be 12.3% (about 0.5 million Singaporeans). Risk factors associated with SDE were found to be age, gender, ethnicity, hypertension and contact lens wear. Smoking was not associated with SDE.

The main part of this thesis sought to evaluate the efficacy of ocular thermography in diagnosing DED. A new infrared detector (NEC Thermo Tracer TH 9260) with relatively high resolution was used. Inter-image, inter-occasion and inter-examiner repeatability was first studied on 21 healthy and 15 DED subjects. Ocular surface marking and ocular surface temperature (OST) acquisition was performed with a novel 'diamond' method using a custom-designed OST analysis V2 software. Ten out of the twelve tested OST indices were shown to be highly repeatable for three studied time points: 0 s, 5 s and 10 s. They were temperatures of the geometric center of the cornea (GCC), mean temperature (MOST) of the region of interest (ROI), maximum (MaxT) and minimum (MinT) temperatures of the ROI, extreme temporal (T1) and nasal conjunctiva (T4), mid temporal (CT) and nasal conjunctiva (CN) and temporal (LT) and nasal limbal (LN).

Another 62 DED and 63 age- and sex-matched controls were then recruited and the ten static and dynamic OST indices were evaluated. Static measures were study of absolute OST at t = 0 s, 5 s and 10 s after eye opening. Dynamic measures were study of mean change and net change in OST over 10 s of sustained eye opening. Static measures on eight OST indices (GCC, MOST, MinT, MaxT, T4, CT, LT and LN) at t = 0 s, 5 s and 10 s and dynamic measures on two OST indices (T4 at 3 s onward and MaxT at 5 s onward) were found to be valuable in detecting DED. The temperature metrics (static and dynamic) were identified for further investigation. Thereafter, the diagnostic ability of the temperature metrics were evaluated singly and as combinations in terms of their area under the curve (AUC), Youden index and discrimination power. Receiver operating characteristic curves were plotted for each metric. Best detectors for DED were found to be the T4 temperature metrics: particularly T4-5 and T4-10 (i.e. absolute temperature of the extreme nasal conjunctiva at 5 s and 10 s). Values of T4-5 of < 34.8 °C were found to give sensitivity and specificity of 87.1% and 50.8% respectively and values of T4-10 of < 34.6 °C were found to give sensitivity and specificity of 77.6% and 61.9% respectively. The two temperature metrics had highest Youden index as compared to other metrics and were shown to be useful in view of AUC > 70% but of limited performance in view of their discrimination power. Nevertheless, measuring T4-5 and T4-10 was found to be comparable to other conventional methods for DED. T4-10 was better than T4-5 in view of higher AUC and Youden index. None of the tested dynamic metrics was good detector for DED and combining metrics were not able to increase the diagnostic ability.

The last part of this thesis was to validate the effectiveness of some common conventional dry eye tests, to study their correlation with T4 temperature metrics and derive the best composite/combined tests for DED. Sixty two DED patients and 82 controls were studied. The conventional clinical tests examined were: symptom evaluation using McMonnies dry eye questionnaire (Mscore) and symptom count (Scount), fluorescein break-up time (FBUT) and corneal epithelial staining (CES), non-invasive break-up time (NIBUT) and tear meniscus height (TMH). Mscore and Scount was the best detectors for DED, followed by FBUT and CES. Discordance between signs and symptoms for DED was further confirmed. Combining CES with T4-10 (series) can be future objective tests for DED. Further research is warranted, particularly to (1) validate the ability of T4-10 as a stand-alone test for DED and (2) work out an algorithm and validate the diagnostic ability of the recommended combined test (CES and T4-10) using newly recruited subjects.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Thesis Format

This thesis is presented in ‘Alternative Format’. The decision to present the thesis this way was taken as several of the chapters featured here had already been either published, or prepared for submission to peer-reviewed journals. Where manuscripts based on these chapters have been published, or submitted for publication in a refereed journal it is indicated on the first page of the chapter. The author’s contribution to the work presented in each chapter is also identified on the first page of each chapter.

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1 Introduction

The International Dry Eye workshop (DEWS) defined dry eye as a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and may also be accompanied with inflammation of the ocular surface (DEWS, 2007a).

Dry eyes can be classified into aqueous tear-deficient and lipid-deficient evaporative dry eye (Lemp, 1995, DEWS, 2007a) (Fig. 1.1). Aqueous-deficient dry eye (ADDE) is subdivided into Sjögren syndrome dry eye and non-Sjögren syndrome dry eye. Patients with ADDE present with failure of lacrimal gland secretion. It can be due to lacrimal acinar cells destruction or dysfunction. This in turn causes tear hyperosmolarity, because of a reduced aqueous tear pool. Tear film hyperosmolarity causes hyperosmolarity of the ocular surface epithelial cells and stimulates inflammatory events (Nelson et al., 1983, Pflugfelder et al., 1997). Evaporative dry eye is due to excessive tear loss from the exposed ocular surface with normal lacrimal secretory functions. It can be further described as intrinsic and extrinsic. Intrinsic is due to intrinsic disease affecting lid structures or dynamics whereas extrinsic is due to increase evaporation by their pathological effects on the ocular surface. Intrinsic factors include meibomian gland dysfunction (MGD), disorders of lid aperture and lid/globe congruity or dynamic and low blink rate. Extrinsic factors include ocular surface disorders, contact lens wear, ocular surface disease, and allergic conjunctivitis (Lemp, 2005). The most common type of intrinsic dry eye is MGD. There are many types of causes leading to MGD such as acne rosacea, seborrheic dermatitis, and atopic dermatitis. The most common type of extrinsic factor is ocular surface disorder.
Dry eye symptoms can be debilitating and affect psychological health and overall sense of well-being. In addition, dry eye leads to increased risk of infection (Lemp, 1998). There is no known cure for dry eye, although there are a number of symptomatic relief treatments available. The total annual healthcare cost ranged from US $0.27 million in France to US $1.10 million in the UK for every 1,000 patients managed by ophthalmologist (Clegg et al., 2006). Anecdotal conversations with the eye doctors in Singapore suggest that the number of dry eye patients seeking for treatment in the hospitals has also increased drastically. However, there is lack of published data on the prevalence of dry eye in Singapore.

Assessment and diagnosis of dry eye is still a challenging task in many cases (Savini et al., 2008). Contributing factors that warrant further attention may include firstly, the invasiveness and low degree of standardization of most of the conventional tests (eg. Schirmer, TBUT and ocular surface staining); secondly, incomplete knowledge about the pathophysiology of the tests (eg. TBUT) and thirdly, the overlapping of dry eye symptoms with those of other conditions, such as conjunctivochalasis (which induce unstable tear film) or delayed tear clearance (which cause ocular
irritation). It was suggested that future studies in dry eye diagnosis should take into account of both subjective and objective parameters (Savini et al., 2008).

It was evident that ocular thermography has the capability in detecting early ocular inflammation as well as dry eye (Morgan et al., 1993, Morgan et al., 1995, Fujishima et al., 1996, Morgan et al., 1996, Mori et al., 1997, Craig et al., 2000, Singh and Bhinder, 2003, Singh and Bhinder, 2005, Zelichowska et al., 2005, Kamao et al., 2011, Su et al., 2011). This study was designed to evaluate the ability of ocular thermography in assessing tear film and dry eye.

1.1 Overview of Tear Film Structure

Traditionally, tear film has been described as a trilaminar structure, consisting of lipid, watery aqueous and mucin layers (Wolff, 1946, Holly and Lemp, 1977) (Fig. 1.2). The thickness of human tear film was estimated to be between 4 µm (Benedetto, 1975) and 8 µm (Ehlers, 1965), measured using invasive tests. Prydal et al. (1992) reported a value of 40 µm, measured using confocal microscope and interferometry. It was proposed that the extra thickness was contributed by deeper, denser layers of mucus, which was underestimated previously. Some animal studies on a more recent estimation of tear film thickness were far lower. For examples, Chen et al. (1997) reported a range of 2-6 µm in rats and Tran et al. (2003) reported an average of 7 µm in mice; measured with electron microscope. King-Smith et al. (2000) and Wang et al. (2003) suggested that human tear film was approximately 3 µm, measured with reflectance spectra and optical coherence tomography respectively.

![Figure 1.2. The structure of tear film (redrawn from Holly and Lemp, 1977).](image)
The structure of the tear film is complex and difficult to be determined because conventional chemical fixation disrupts its morphology (Johnson and Murphy, 2004). The film was first reported as a substantial free-fluid layer, which was later reported as a homogenous, fine network-like structure throughout the tear film in rats (Chen et al., 1997) and mice (Tran et al., 2003) using electron microscopy following in vivo cryofixation with freeze substitution. Tran et al. (2003) also reported a single tear phase on the murine tear film when sampled at a spatial resolution of 1 µm. It was questionable on whether the tear film structure of rodents was representative of human tears and their higher tear stability and lower blinks rate warrant caution in applying the findings to human eye (Duke-Elder, 1968).

A more recent study investigating the surface lipid layer using grazing incidence x-ray diffraction technique demonstrated the existence of two-dimensional order in mammalian pre-ocular tear film. It was suggested that the two-dimensional ordering is set by generic lipid-lipid interactions. The study further supported the previous hypothesis that tear film has a layered structure (Petrov et al., 2007).

1.2 Dry Eye Assessment: Conventional Tests

Due to the multifactorial nature of tear film disorders it is important to conduct a comprehensive evaluation with multiple tests on dry eye. The 1995 National Eye Institute (NEI) / Industry workshop defined global characteristics of dry eye as corneal staining (Grade 0-3 for 5 areas) > 3 out of 15 with fluorescein, conjunctival staining (Grade 0-3 for 6 areas-van Bijsterveld scheme) > 3 out of 18 with Rose Bengal or Lissamine Green, tear osmolarity > 312 mOsm/l, and fluorescein break-up time ≤ 10 s (Lemp, 1995, Johnson and Murphy, 2004). Although tear hyperosmolarity is mentioned as part of the criteria for diagnosis, measuring it is currently impractical in the clinical setting (Korb, 2000).

Whilst there are many tests for dry eye, there remains a great disparity among the symptoms and signs in many dry eye patients. Determining the cause of dry eye when minimal clinical signs are present is difficult and the diagnosis is complicated further when there is a lack of correlation between symptoms and objective tests (Nelson et al., 2000). In an attempt to overcome these problems and to standardize the diagnostic criteria for dry eye, the Diagnostic Methodology Subcommittee of the international Dry Eye Workshop (DEWS) has recommended that if a battery of tests is to be performed for screening and diagnosing dry eye, it should be in the following sequence that best preserves their integrity (Table 1.1) or in the practical test sequence (Table 1.2):-
<table>
<thead>
<tr>
<th>Group</th>
<th>Assessment</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Clinical history</td>
<td>Questionnaire</td>
</tr>
<tr>
<td></td>
<td>Symptoms eg, dry eye</td>
<td>Symptom questionnaire</td>
</tr>
<tr>
<td>B</td>
<td>Evaporation rate</td>
<td>Evaporimetry</td>
</tr>
<tr>
<td>C</td>
<td>Tear Stability</td>
<td>Non-Invasive TF BUT (or NIBUT)</td>
</tr>
<tr>
<td></td>
<td>Tear lipid film thickness</td>
<td>Interferometry</td>
</tr>
<tr>
<td></td>
<td>Tear meniscus radius/volume</td>
<td>Meniscometry</td>
</tr>
<tr>
<td>D</td>
<td>Osmolarity; proteins lysozyme; lactoferrin</td>
<td>Tear sampling</td>
</tr>
<tr>
<td>E</td>
<td>Tear stability</td>
<td>Fluorescein BUT (FBUT)</td>
</tr>
<tr>
<td></td>
<td>Ocular surface damage</td>
<td>Grading staining fluorescein; lissamine green</td>
</tr>
<tr>
<td></td>
<td>Meniscus, height, volume</td>
<td>Meniscus slit profile</td>
</tr>
<tr>
<td>F</td>
<td>Tear secretion turnover</td>
<td>Fluorimetry</td>
</tr>
<tr>
<td>G</td>
<td>Casual lid margin oil level</td>
<td>Meibometry</td>
</tr>
<tr>
<td>H</td>
<td>Tear secretion</td>
<td>Schirmer I with anaesthesia</td>
</tr>
<tr>
<td></td>
<td>“Reflex” tear secretion</td>
<td>Schirmer I without anaesthesia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Schirmer II (with nasal stimulation)</td>
</tr>
<tr>
<td>I</td>
<td>Signs of MGD</td>
<td>Lid (meibomian gland morphology)</td>
</tr>
<tr>
<td>J</td>
<td>Meibomian gland dysfunction</td>
<td>MG expression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expressibility of secretions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Volume</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quality</td>
</tr>
<tr>
<td>K</td>
<td>Ocular surface damage</td>
<td>Rose Bengal stain</td>
</tr>
<tr>
<td>L</td>
<td>Meibomian tissue mass</td>
<td>Meibography</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**Table 1.2.** A practical sequence of tests (adapted from DEWS, 2007a).

- Clinical history
- Symptom questionnaire
- Fluorescein BUT (FBUT)
- Ocular surface staining grading with fluorescein / yellow filter
- Schirmer I test without anaesthetic, or I with anaesthetic, and/or Schirmer II with nasal stimulation
- Lid and meibomian morphology
- Meibomian expression
- Other tests may be added according to availability

It was generally well agreed that the currently available clinical tests serve different purposes: both quantitatively and qualitatively. For examples, symptom assessment is useful as a subjective measurement for dry eye. Schirmer tests, phenol red thread test (PRT) and tear meniscus height (TMH) are normally used as battery of tests to reflect aqueous production/tear volume. The tear stability / dynamic can be tested using fluorescein break-up time (FBUT) and non-invasive break-up time (NIBUT). The ocular surface damaged tested by graded fluorescein or lissamine green staining (Kaercher and Bron, 2008). In addition, tear ferning test (TFT) can be used to assess the tear film integrity and quality (Rolando, 1984).
1.2.1 Symptom Assessment using dry eye questionnaires

Although there are different causes of dry eye, the symptoms reported are generally similar and not specific to each individual subtype. These symptoms include grittiness, foreign body sensation, burning, soreness, stinging, dryness, blurry vision, reflex tearing and photophobia (Baum, 1985, Whitcher, 1987, McMonnies, 1986, McMonnies and Ho, 1987, Nichols et al., 1999). Therefore it is crucial that evaluation of dry eye should include an assessment of subjective symptoms. Dry eye questionnaires are used to screen individuals for the diagnosis of dry eye, to assess the effects of treatments or to grade disease severity. Questionnaires can be used for population-based studies or to study the natural history of disease (DEWS, 2007b). When the terms “dry eye” and “questionnaire” were searched in PubMed and limits of “English language” and “human” were applied, a list of questionnaires were found and shown in Table 1.3a and 1.3b in terms of their summary and description/use.

Table 1.3a. Symptoms and quality of life instruments (adapted from DEWS, 2007b).

<table>
<thead>
<tr>
<th>Questionnaire/description</th>
<th>Questionnaire Summary</th>
<th>Description/Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>McMonnies Key questions in a dry eye history (McMonnies, 1986)</td>
<td>15 questions</td>
<td>Screening questionnaire— used in a clinic population</td>
</tr>
<tr>
<td>McMonnies Reliability and validity of McMonnies Dry Eye Index. (Nichols et al., 2004b)</td>
<td>Previously described</td>
<td>Screening questionnaire Dry eye clinic population</td>
</tr>
<tr>
<td>CANDEES A patient questionnaire approach to estimating the prevalence of dry eye symptoms in patients presenting to optometric practices across Canada (Doughty et al., 1997)</td>
<td>13 questions</td>
<td>Epidemiology of dry eye symptoms in a large random sample</td>
</tr>
<tr>
<td>OSDI The Ocular Surface Disease Index (Schiffman et al., 2000)</td>
<td>12-item questionnaire</td>
<td>Measures the severity of dry eye disease; end points in clinical trials, symptoms, functional problems and environmental triggers queried for the past week</td>
</tr>
<tr>
<td>OSDI and NEI-VFQ comparison (Vitale et al., 2004)</td>
<td>Comparison of existing questionnaires</td>
<td>Tested in Sjogren Syndrome population</td>
</tr>
<tr>
<td>IDEEL Comparing the discriminative validity of two generic and one disease-specific health-related quality of life measures in a sample of patients with dry eye (Rajagopalan et al., 2005)</td>
<td>3 modules (57 questions): Daily Activities Treatment Satisfaction Symptom Bother</td>
<td>Epidemiologic and clinical studies</td>
</tr>
<tr>
<td>Salisbury Eye Evaluation Relation between signs and symptoms of dry eye in the elderly (Schein et al., 1997)</td>
<td>Standardized 6-question questionnaire*</td>
<td>Population-based prevalence survey for clinical and subjective evidence of dry eye</td>
</tr>
<tr>
<td>Salisbury Eye Evaluation Self-reported assessment of dry eye in a population-based setting (Bandeen-Roche et al., 1997)</td>
<td>Standardized 6-question questionnaire*</td>
<td>Population-based prevalence survey for clinical and subjective evidence of dry eye</td>
</tr>
<tr>
<td>Dry Eye Epidemiology Projects (DEEP) Sensitivity and specificity of a screening questionnaire for dry eye (Oden et al., 1998)</td>
<td>19 questions</td>
<td>Screening</td>
</tr>
<tr>
<td>Questionnaire/description</td>
<td>Questionnaire Summary</td>
<td>Description/Use</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Women’s Health Study questionnaire Prevalence of dry eye syndrome among US women (Schaumberg et al., 2003)</td>
<td>3 items from 14-item original questionnaire</td>
<td>Women’s Health Study/ Epidemiologic studies</td>
</tr>
<tr>
<td>National Eye Institute Visual Function Questionnaire (NEI-VFQ) (Mangione et al., 1999)</td>
<td>25-item questionnaire: 2 ocular pain subscale questions</td>
<td>Useful tool for group-level comparisons of vision-targeted, health-related QOL in clinical research; not influenced by severity of underlying eye disease, suggesting use for multiple eye conditions.</td>
</tr>
<tr>
<td>Dry Eye Questionnaire (DEQ) Habitual patient-reported symptoms and clinical signs among patients with dry eye of varying severity (Begley et al., 2003)</td>
<td>21 items on prevalence, frequency, diurnal severity and intrusiveness of symptom</td>
<td>Epidemiologic and clinical studies</td>
</tr>
<tr>
<td>Dry Eye Questionnaire (DEQ) Use of the dry eye questionnaire to measure symptoms of ocular irritation in patients with aqueous tear deficiency dry eye (Begley et al., 2002)</td>
<td>As above</td>
<td>As above</td>
</tr>
<tr>
<td>Contact Lens DEQ Responses of contact lens wearers to a dry eye survey (Begley et al., 2000)</td>
<td>13 questions</td>
<td>Screening questionnaire for dry eye symptoms in contact lens wearers</td>
</tr>
<tr>
<td>Melbourne Visual Impairment Project The epidemiology of dry in Melbourne, Australia (McCarty et al., 1998)</td>
<td>Self-reported symptoms elicited by interviewer-administered questionnaire</td>
<td>Epidemiologic studies</td>
</tr>
<tr>
<td>National Eye Institute 42-Item Refractive Error Questionnaire (Hays et al., 2003)</td>
<td>42-item questionnaire: 4 related questions: ocular pain or discomfort, dryness, tearing, soreness or tiredness</td>
<td>QoL due to refractive error</td>
</tr>
<tr>
<td>Sicca/SS questionnaire Validation of the Sicca symptoms inventory for clinical studies of Sjogren’s syndrome (Bowman et al., 2003)</td>
<td>Inventory of both symptoms and signs of Sjogren Syndrome</td>
<td>Epidemiologic studies for Sjogren Syndrome</td>
</tr>
<tr>
<td>Bjerrum questionnaire Study Design and Study Populations (Bjerrum K, 2000a)</td>
<td>8-part questionnaire which includes an ocular part with 14 questions</td>
<td>QoL due to SS dry eye, diagnosis of dry eye, epidemiology of SS</td>
</tr>
<tr>
<td>Bjerrum questionnaire Dry Eye Symptoms in patients and normal (Bjerrum K, 2000b)</td>
<td>As above</td>
<td>Screening questionnaire</td>
</tr>
<tr>
<td>Bjerrum questionnaire Test and symptoms in keratoconjunctivitis sicca and their correlation (Bjerrum K, 1996)</td>
<td>Dry eye tests Ocular symptom questionnaire (14 questions)</td>
<td>Examine correlation between dry eye test and ocular symptom questionnaire responses</td>
</tr>
<tr>
<td>Utility assessment questionnaire Utility assessment among pts with dry eye disease (Schiffman et al., 2003)</td>
<td>Utility assessment</td>
<td>Utility assessment</td>
</tr>
<tr>
<td>Japanese dry eye awareness study Results of a population-based questionnaire on the symptoms and lifestyles associated with dry eye (Shimmura et al., 1999)</td>
<td>30 questions relating to symptoms and knowledge of dry eye</td>
<td>Population-based, self-diagnosis study to assess public awareness and symptoms of dry eye</td>
</tr>
<tr>
<td>Sicca/SLE questionnaire Oral and ocular sicca symptoms and findings are prevalent in systemic lupus erythematosus (Jensen et al., 1999)</td>
<td>8-question symptom questionnaire</td>
<td>Screening for dry eye symptoms in SLE patients</td>
</tr>
<tr>
<td>American-European Consensus Group Classification criteria for Sjogren’s syndrome: a revised version of the European criteria proposed by the American-European Consensus Group (Vitali et al., 2002)</td>
<td>6 areas of questions: Ocular symptoms; oral symptoms; ocular signs; histopathology; oral signs; auto-antibodies</td>
<td>Clarification of classification of primary and secondary Sjogren syndrome, and of exclusion criteria.</td>
</tr>
<tr>
<td>The Eye Care Technology Forum Impacting Eye Care (Ellwein, 1994)</td>
<td>Issues: Standardizing clinical evaluation</td>
<td>Decree for change</td>
</tr>
</tbody>
</table>
The questionnaires varied in length, intended use, population in which they were tested, mode of administration (self, interviewer, and phone) and extent of validation. Items commonly included were queries related to: clinician-based or other diagnosis of dry eye; frequency and/or intensity of symptoms; effect of symptoms on activities of daily living; effect of environmental triggers on symptoms; presence of dry mouth; effect of visual tasks on symptoms (e.g., computer use); effect of treatment on symptoms; contact lens wear; medications; and allergies. Items infrequently included were queries related to the use of drops, arthritis, thyroid disease, dry nose or vagina, emotional triggers, and global assessment by the patient (DEWS, 2007b).

1.2.2 McMonnies dry eye questionnaire

The Charles McMonnies Dry Eye Questionnaire (DEQ) is a self-administered questionnaire consisting of 12 questions. It was found to be able to discriminate normal and sicca/dry eye syndrome patients with sensitivity reportedly varying between 87% and 98% and specificity between 87% and 97% (Golding and Brennan, 1993, McMonnies and Ho, 1987, McMonnies et al., 1998). Variation in estimates of sensitivity could be due to differences in experimental population, criteria used for dry eye classification and different scoring methods as well as variation in cut off scores ranging from 8 to 19 (McMonnies and Ho, 1987, McMonnies et al., 1998, Gothwal et al., 2010).

Nevertheless, McMonnies DEQ was shown to have the potential of identifying established from marginal cases of ocular sicca, and to obviate the need for additional examination procedures for majority of patients (Morris and Schmid, 1984, McMonnies, 1986). Further analysis has shown that over-45-year-old females can be regarded as representative of optometric patients of both sexes and all adult ages and apart from having application in the routine detection of sicca conditions, it can be of value in detecting ocular sicca symptoms due to contact lenses. The McMonnies DEQ was also reported to be able to identify individuals who are at risk for developing dry eye problems at a later time because of its questioning of the individual's exposure to provoking factors (McMonnies and Ho, 1987) as it includes questions regarding patient history.

McMonnies DEQ has been regarded as the "gold standard" for examining dry eye symptoms in disease conditions. This was supported by (Erickson et al., 2002) who proved that McMonnies DEQ was statistically reliable and provided a consistent and repeatable measurement. However, (Nichols et al., 2004a) reported that it was fairly reliable and valid as a patient reported instrument for use in patient care and clinical studies for dry eye with sensitivity and specificity of 82% and 36% respectively using a cutoff score of 14.5.

In summary, McMonnies DEQ has the following features:

- 12 items- most dichotomous yes/no, weighted scoring
• Screening, used in dry eye clinic population
• Includes age, sex, contact lens wear
• Previous diagnosis of dry eye, triggers (environment, swimming, alcohol)
• Frequency of symptoms: dryness, grittiness, soreness, redness, tiredness (Answers: Never, sometimes, often, constantly)
• Medications, arthritis, dry mouth, thyroid status

1.2.3 Ocular Surface Damage

Ocular surface damage can be assessed using various vital dyes such as fluorescein, Rose Bengal and Lissamine green. Each dye has different properties such that it either pools in the epithelial defects (fluorescein), interacts with an impaired mucin layer on the epithelial surface (Rose Bengal) or stains dead and devitalised cells (Lissamine green) (DEWS, 2007b). Fluorescein is used for the standard assessment for ocular surface damage, with Rose Bengal and Lissamine green reserved for more severe dry eye cases (Bron et al., 2003). It is, however, possible to detect and score corneal and conjunctival staining together, using fluorescein alone if it is viewed through a yellow barrier filter (eg., Wratten 12) (Nichols et al., 2004a).

Dry eye provokes a cascade of cellular reactions, which involves apoptotic cell death and inflammation (Fujihara et al., 1999). This deteriorates the ocular surface protective mechanisms, alters the three tear layers, and causes continuous cellular degeneration of the conjunctiva and corneal epithelium. Once dry eye has developed, inflammation becomes the key mechanism of ocular surface injury, as both the cause and consequence of cell damage (Baudouin, 2001).

Fluorescein and Rose Bengal staining are highly sensitive for dry eye diagnosis (Whitcher, 1987). The fluorescein dye swiftly diffuses into the stromal where the intercellular junctions are disrupted because of the tear constituents’ inability to block the dye (Feenstra and Tseng, 1992).

Three staining schemes for ocular surface are in current use. They are the van Bijsterveld system (VanBijsterveld, 1969), the Oxford system (Bron et al., 2003), and a standardized version of the National Eye Institute (NEI) / Industry Workshop system reported by Lemp (1995a), which is also known as the Lemp’s Scale. The Lemp’s scale has been one of the most commonly used staining schemes for cornea.

1.2.4 Tear Film Stability: FBUT and NIBUT

The tear film constantly changes and undergoes reformation immediately after a blink. It then exhibits a period of stability and finally breaks up provided the eye is left open for a sufficient period of time (Tsubota, 1998). The fluorescein break-up time (FBUT) devised by (Norn, 1969) and (Lemp and Holly, 1970) has been widely used to study the tear film stability. It is also denoted as tear break-up time (TBUT) in some reports. This technique utilizes fluorescein to stain the tears and the
The first appearance of a dark spot after a complete blink is defined as FBUT (Lemp and Hamill, 1973). A reference value of less than 10 seconds is used to identify dry eye (Golding and Brennan, 1993). Significant correlation has been found between ocular surface discomfort and tear break-up time in dry eye subjects (Nally et al., 2000).

TBUT has been reported to have poor repeatability and multiple measurements were recommended (Cho et al., 1992, Cho and Brown, 1993, Nichols et al., 2004a). Studies have also suggested that TBUT results were highly not reproducible (Vanley et al., 1977). It was hypothesized that the instillation of fluorescein itself was a possible cause for the variation in results by destabilizing the tear film (Holly, 1981).

A non-invasive break-up time (NIBUT) was then recommended. NIBUT observes and images the specular image of a pattern projected onto the tear film. Mengher et al. (1985) measured NIBUT using an illuminated rectangular grid pattern that projected onto the cornea surface and viewed through slit lamp (xeroscope) whereby the time for a distortion of the grid to appear reflects the tear break-up time (Mengher et al., 1985). Subsequently, other instruments have been used to measure the NIBUT such as the tearscope Plus (Keeler Inc., London, UK) and other prototypes (Nichols et al., 2002, Yokoi and Komuro, 2004, Wang et al., 2005, Kaercher and Bron, 2008). NIBUT was proven to be reliable in normal subjects (Cho, 1993) but with considerable between-examiner variability (Cho and Douthwaite, 1995, Nichols et al., 2002).

The tearscope also has the capability to quantify the lipid layer thickness through interference pattern (Guillon, 1998, Kaercher and Bron, 2008). Evaluation of the interference patterns and colors of the anterior surface of the tear film lipid layer aids in the diagnosis of the cause of dry eye. Colors indicate a thick and desirable lipid layer and their presence suggests that dry eye disorder is not present (blue = 150 – 180 nm, red/ brown = 120 – 135 nm, yellow = 90 – 105 nm). If only white or shades of grey (particularly if the white or grey is uniform) are observed, the lipid layer is thin (≤ 60 nm) and the probability of dry eye disorder is high. According to (Guillon, 1998), the stability of the tear film was influenced by the nature of the lipid layer, with greater stability being achieved when the lipid layer was thick. This was supported by (Craig and Tomlinson, 1997), who reported that tear evaporation was increased 4 times when the tear film was unstable, if the lipid layer was minimal or confluent.

Mengher et al. (1986) reported a sensitivity of 82% and specificity of 87% for NIBUT as a diagnostic tool for dry eye. Cho (1993) further described the NIBUT technique to be a potential replacement for the conventional TBUT method. It was found that TBUT and NIBUT were poorly correlated (Cho and Douthwaite, 1995).

There were other technologies not primarily developed for this purposes has also been used to assess the tear film stability. The TMS-1 corneal topographer, for instance, has been used to
differentiate dry eye from non-dry eye (Liu and Pflugfelder, 1999). Later another group of researches performed a consecutive measurement of corneal topography to measure tear film build-up time for normal and dry eye patients (Nemeth et al., 2002). More recently the Tear film Stability Analysis System (TSAS) was developed to allow clinician to record consecutive topographic images every second for 10 second and study the tear film stability. In dry eye patients, a gradual increase in SRI (surface regularity index) and SAI (surface asymmetry index), a reduction in TMS-BUT (time for the ocular surface to change its refractive power by 0.5 diopters after each blink) and higher values of TMS-BUA (the area where the break-up time is less than or equal to 5 seconds) have been reported (Goto et al., 2003, Kojima et al., 2004). The technique was described to be of potential in screening dry eye patients especially the mild cases but however, the definition of normal values in different age groups and its diagnostic criteria are still lacking (Savini et al., 2008).

1.2.5 Tear Osmolarity

Tear film hyperosmolarity may reasonably be regarded as the signature feature that characterizes the condition of “ocular surface dryness” (DEWS, 2007a). The recommended cut-off value of 316 mOsm/l is well validated as diagnostic for dry eye (Tomlinson et al., 2006). Although the measurement of tear osmolarity (TearLab, TearLab corporation, San Diego, CA, USA) has been offered as a “gold standard” for dry eye diagnosis (Farris, 1994), this test is generally not done in the clinical setting as it require expert technical support and specialized laboratories.

A multicenter, 10-site study with recruitment of 314 subjects aged 18 to 82 was done to evaluate the use of tear osmolarity in the diagnosis of dry eye and shown positive results (Lemp et al., 2011). Tear osmolarity was reported to have relatively high sensitivity (73%) and specificity (92%) as compared to other tests (54% for corneal staining, 60% for conjunctival staining and 61% for meibomian gland grading on sensitivity; 45 % for TBUT and 51% for Schirmer test on specificity). There were inter-ocular differences in osmolarity correlated with increase dry eye severity.

1.2.6 Tear Ferning Test

Tear Ferning Test (TFT) is done by taking a tear sample (1 - 2 μl) from the lower tear meniscus and applied to a glass slide. When the tears evaporated, a characteristic ferning pattern developed due to crystallisation of the tears. The ferning pattern is influenced by the protein and electrolyte composition of the tears and this pattern can be viewed under the microscope and used as an index for dry eye (Rolando, 1984). In dry eye, the delicate fronded pattern becomes broken up and irregular. Rolando (1984) reported four types of fern category (Type I, II, III and IV) which is categorized according to their presence, size and density of the ferns seen. He reported that normal tear film usually show a Type I or Type II fern pattern (with many ferning). On the other hand, Type III and Type IV fern pattern (with little or absence of ferning) is usually associated with dry eye patients.
Intra- and inter-observer repeatability of the Rolando grading system was reported to be 85.4 % and 92.1 % (Pensyl and Dillehay, 1998). Although the Rolando grading scale (Rolando, 1984) is the most often used, it was not introduced for its purpose originally and there remains no clearly defined protocol. Recently, a new five-point Masmali grading scale (Grades 0-4) has been developed to overcome some of the limitations associated with Rolando scale, such as overlap between Type I and II grades (Masmali et al., 2014). Besides discrimination, the new grading scale was found to be linear and reliable (Masmali et al., 2014). The grading scale was then applied on tear samples of eighty subjects (40 dry eyes and 40 non-dry eyes) aged 19 to 53 years using 0.1 increments and concluded that it has good validity in differentiating these subject types. It was recommended that Grade ≥ 2 can be classified as abnormal patterns (Masmali et al., 2015).

### 1.2.7 Meibomian Gland Evaluation

Meibomian gland dysfunction is a highly prevalent subtype of dry eye (Albietz, 2000). A decrease of meibomian oil caused increased tear evaporation and ocular surface damage due to increased osmolarity (Murphy et al., 2001). When viewing the eye under slit lamp biomicroscope, capping of the orifices, cheesy secretion on expression and frothing of the eyelid margin indicates meibomian gland dysfunction. Concretions can be associated with this condition. This can lead to excessive evaporation of the aqueous component of the tears and treating the aspect is often a component of dry eye management, as aqueous deficiency and excessive tear evaporation often coexist.

#### 1.2.7.1 Meibometry

This is a quantitative measurement of the basal level of meibomian lipid whereby lipid is blotted onto a plastic tape, which produces a strip of increased transparency. The amount of transparency can then be quantified and reflects the lipid uptake index, using a photometer (Chew et al., 1993).

#### 1.2.7.2 Meibography

Meibography involves the transillumination of the meibomian glands after lid eversion. Meibomian glands will be silhouette and any missing or ‘drop-out’ of the glands can be quantified. However, the manipulation of the lids could stimulate reflex tearing (Mathers et al., 1991).

### 1.2.8 Tear Flow/Volume Testing

The simple assessment of the tear meniscus can combined with either the phenol red thread or Schirmer test for the assessment of tear flow / volume (Albietz, 2000).

#### 1.2.8.1 Assessment of the Tear Meniscus

Small tear volumes may result in dry eye symptoms, especially in ADDE (Mainstone et al., 1996, Golding et al., 1997, Oguz, 2008). Holly (1981) has shown that the tear meniscus represented more than 70% of the tear volume. Guillon and Guillon (1988) reported that the tear meniscus contained 90% of the total exposed tear volume. A diminished tear meniscus height (TMH) is
therefore represents a reduced tear volume. Measuring TMH was proven to be a useful test for dry eye (Mainstone et al., 1996) and well correlated with tear volume.

TMH can be measured by placing a short slit onto the lower meniscus and viewed using a graticule eyepiece or pachometer in conjunction with a slit lamp biomicroscope or using photography. Since there is no standard method in measuring TMH, different cut-off values have been suggested for dry eye diagnosis. Mainstone et al. (1996) reported a sensitivity of 93% and specificity of 67% for TMH at the cut off of 0.35 mm. Golding et al. (1997) who used photographic method for evaluating the TMH, reported a higher TMH value comparable to the findings by Mainstone et al. (1996). Kwong and Cho (2001) reported a sensitivity of more than 80% for the cut off for TMH of 0.21 mm with marginal repeatability for the Hong Kong Chinese. A study on Japanese subjects reported a sensitivity of 89% and specificity of 78% at the cut off for meniscus radius of 0.25 mm (Yokoi et al., 2004). A more recent study further validated the sensitivity and specificity (80.56% and 89.33% respectively) of measuring lower tear meniscus using Spectral OCT in diagnosing dry eye syndrome. TMH was also reported to be correlated well with Schirmer test, break-up time, and subjective symptoms (Czajkowski et al., 2012).

The repeatability of TMH can be higher if the measurements were replicated immediately as compared to measurements taken on different days (Johnson and Murphy, 2006). The repeatability can be enhanced by using more advanced technology such as the videography methods as compared to solely using slit lamp biomicroscopy (Nichols et al., 2004a). Shen et al. (2009) has shown that the reproducibility of tear meniscus measurement by Fourier-domain optical coherence tomography (OCT) was better than other OCT instruments.

1.2.8.2 Schirmer Test
The Schirmer test is one of the oldest tests for dry eye diagnosis which is highly invasive and is normally performed later in the diagnostic sequence. The Schirmer paper is placed at the lower lid margin in a non-anaesthetised closed eye for 5 minutes and the length of wetting is measured (Fig. 1.3). A normal tear production will show a measurement of 10 mm or more while a value of less than 5 mm is suggestive of dry eye (VanBijsterveld, 1969). Schirmer I test is a measurement of total (reflex and basic) tear secretion and Schirmer II test measures the reflex tears (Doughman, 1973). To avoid tearing, the Schirmer test has sometime been used with anaesthesia and is said to measure basic tear secretion (Jones, 1966).
Fluctuation in the results is common when the test is done during different visits and by different examiner. Nichols et al. (2004a) reported that there is increased in variability of the Schirmer test results as the Schirmer scores increase. Environmental conditions such as temperature or humidity may affect the reliability of the test. The Schirmer test is in common use especially with severe dry eye patients (Bron, 2002). However, research has suggested that it is neither reliable nor valid (Cho and Yap, 1993). According to Abelson et al. (2009), the clinical value of the Schirmer test had long been controversial, but when performed correctly and interpreted in the context of other diagnostics, the Schirmer test could be of value. It was reported that as many as 15 per cent of normal subjects may fail the test with results of less than 3 mm wetting in 5 minutes.

1.2.8.2 Phenol Red Thread Test

Phenol red thread (PRT) test uses a cotton thread impregnated with phenol red dye to measure tear volume (Patel et al., 1998). Phenol red is pH sensitive and changes from yellow to red when wetted by tears. The thread is about 70 mm long and is normally inserted into the lower fornix for 15 seconds and the wetted length is measured (Fig. 1.4). Patel et al. (1998) reported a sensitivity of 86% and a specificity of 83% using a cut off values of 20 mm (in 120 seconds) to differentiate between aqueous deficient and non-aqueous deficient dry eye. Another study has suggested that an average measurement of less than 11 mm in 15 seconds is considered diagnostic of aqueous-deficient dry eye (Albietz, 2000).
Figure 1.4. Phenol red thread test (image credit: Tan Shun Jing).

Cho and Chan (2003) studied inter-examiner difference and the effect of training on the PRT results in Hong-Kong Chinese, and showed that there was significant variability in the reproducibility of the test. The variability could be reduced after the examiners underwent intensive training. The test was found to be highly repeatable (Tomlinson et al., 2001) but was conflicting with another study done by Nichols et al. (2004a). The sensitivity and specificity of the PRT was reported to be 56% and 69% respectively using a cut off value of 12 mm. Even though PRT has shown some agreement with Schirmer I test, 32% of the study population have discordant results. PRT and Schirmer I can be complementary to each other but further studies were recommended to validate their usefulness in clinical practice (Labetoulle et al., 2002).

1.3 Challenges in Dry Eye Diagnosis

It was evident that the diagnostic values of the currently available clinical tests are inconclusive (Goren and Goren, 1988, Kallarackal et al., 2002, Begley et al., 2003). Saleh et al. (2006) reported that the diagnostic value of evaluating dry eyes has not been repeatable and reliable because of variable results, poor reproducibility and low sensitivity. It has been documented that especially in moderate/mild dry eye; diagnostic tests were prone to give conflicting results. For example, (Kallarackal et al., 2002) reported that there was a poor correlation between Schirmer’s test and FBUT in dry eye patients. Begley et al. (2003) found that dry eye symptoms were not correlated with ocular surface staining and on the other hand, Moore et al. (2009) found a poor correlation between dry eye symptoms and phenol red thread test. It was well understood that the eye care practitioners are mostly relying on battery of tests to diagnose dry eye. Nichols et al. (2000) reported in their retrospective study that 43.7% of the eye care practitioners combined symptom assessment with fluorescein corneal staining in diagnosing dry eye although further investigations showed that the symptoms of dry eye did not associated with clinical test findings conducted (meibomian gland assessment, TMH, TBUT, fluorescein and rose bengal staining, PRT and Schirmer test (Nichols et al., 2004b).
In view of lack of gold standard in diagnosing dry eye, Brewitt and Sistani (2001) reported the immense need at both the national and international level to standardize terminology and diagnostic tests. They claimed that as the prevalence of dry eye disease will increase with aging population, every dry eye patient has the right to expect the state-of-the-art therapy and education on how to manage the condition in the best possible way. Savini et al. (2008) described the challenges of dry eye diagnosis. It was recommended that non-invasive tests are more favourable despite its cost and complexity and besides exploring for new test; researches could look into combination of tests. Yokoi et al. (2004) proved the usefulness of meibography and meibometry in screening meibomian gland dysfunction in detecting severe cases and claimed that modern computer-assisted information technology could aid in precise diagnosis.

The more recent studies were exploring into biomarker for dry eye and it was reported that tear osmolarity could be the best single metric to diagnose and classify dry eye disease (Lemp et al., 2011) and could be a potential objective biomarker to diagnose dry eye (McGonnigle et al., 2012).

According to the Diagnostic Methodology Subcommittee of the international Dry Eye Workshop (DEWS), tests on dry eye are used for a variety of purposes (DEWS, 2007a):

(i) To diagnose dry eye in everyday clinical practice.
(ii) To assess eligibility in a clinical trial (ie., recruitment).
(iii) To follow quantitative changes over the duration of a clinical trial (monitoring). These tests might differ from those used in recruitment, eg., monitor the stimulation of mucin production in a drug study.
(iv) To characterize dry eye as part of a clinical syndrome, eg., Sjögren syndrome.
(v) To follow the natural history of the disorder, eg., dry eye.

When a test is being evaluated for efficacy, the test population may have been classified as affected or non-affected. Similarly, the performance of any “new” test may be compromised when the test is applied on a dry eye population that have been diagnosed using non-established criteria. A protocol is suggested as a model for evaluating diagnostic tests for dry eye (DEWS, 2007a).

(i) The diagnostic test will be applied to a study sample of normal subjects and dry eyes, as defined by symptoms, and the “traditional” ophthalmological tests, for example Schirmer test, TBUT and ocular surface staining.
(ii) The values obtained for the new diagnostic test in the two samples will be determined, frequency distribution of data will be compiled, and an initial cut-off value, distinguishing affected from non-affected, will be set at the intercept of the two frequency curves.
(iii) The sensitivity, specificity, and predictive values of a positive and negative test result and the overall accuracy of the test will be determined for this cut-off value.
(iv) A range of different cut-off values for the test statistic can then be analysed by constructing a receiver-operator characteristics (ROC) curve to maximize the sensitivity and the specificity of the test.

(v) The proposed cut-off value thus determined for the test will then be assessed for its efficacy on a new, independent sample of normal and dry eye subjects. An iterative process may then be required to arrive at a final cut-off value.

The characteristics of various dry eye diagnostic tests currently available and their effectiveness used singly or in combination, can be found in appendix 1.1. The tests included are those for which values of sensitivity and specificity are available in the literature. Predictive values of the listed tests (being positive, negative and overall accuracy) are calculated for a 15% prevalence of dry eye in the population studied.

### 1.4 Infrared Ocular Thermography

A screening test for dry eyes that is objective, non-invasive, and rapid is warranted (Kamao et al., 2011). Although the currently available clinical test could be quick and simple to perform; they tend to be unreliable and may lead to the wrong diagnosis of dry eyes as discussed previously. Some are invasive thus making measuring of tear volume or tear stability not natural. Conducting a battery of tests to help in diagnosing dry eye is time consuming. Ocular thermography has the potential to screen and diagnose dry eye. Using an infrared technique, it can be done instantaneously, non-invasively, does not require anaesthetic drops (Morgan et al., 1995) and could be a single test to diagnose dry eye.

Infrared (IR) ocular thermography is a modern technology used to determine the surface temperature of the eye and pre-orbital skin by measuring the amount of IR radiation emitted from the surface with an infrared thermal imaging camera. Measurements are then processed into a colour coded display graph (thermogram) for interpretation and analysis (Morgan et al., 1993). With current technology, the infrared ocular thermography offers great opportunities for monitoring the temperature of the anterior eye in real time and provides opportunities to study the pathophysiological aspects of the eye.

Temperature has been one of the fundamental parameters of tissue metabolism and the measurement of temperature has long been regarded as a guide to bodily health (Morgan et al., 1993). It is interesting to note that body temperature has a strong positive correlation with ocular surface temperature (OST). For every 1 °C increase in body temperature, there will be almost identical rise in OST (Purslow and Wolffsohn, 2007).
1.4.1 Medical Applications of Thermometry

Thermometry has wide applications in the medical field. The mercury in glass thermometer used to be the standard instrument for measuring body temperature before development of infrared (IR) measurement methods began in the 1920s. Advancements in IR technology developed for military usage after World War II got adapted into the medical field. Since then, IR thermometry has been used in many conditions including breast cancer, deep vein thrombosis of the legs, rheumatism, as well as in research on headache, dental and facial conditions (Morgan et al., 1993).

1.4.2 Background and Evolution of Ocular Surface Temperature measurement

Table 1.5 show how the measurements of ocular surface temperature (OST) evolved, from direct to indirect techniques, from placement of a mercury bulb at the corneal surface to miniaturized thermister bead and eventually using infrared.

Table 1.4. Techniques for measuring human ocular surface temperature (OST) and the key findings (Tan et al. 2009c).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Mean Temp. (°C)</th>
<th>Range (°C)</th>
<th>Inter-ocular temp difference (°C)</th>
<th>Difference b/w limbus and central cornea (°C)</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dohnberg 1876a</td>
<td>36.50-36.70</td>
<td></td>
<td></td>
<td></td>
<td>Mercury bulb</td>
</tr>
<tr>
<td>Galezowski 1877a</td>
<td>36.40</td>
<td></td>
<td></td>
<td></td>
<td>Mercury bulb</td>
</tr>
<tr>
<td>Silex 1893a</td>
<td>35.55</td>
<td></td>
<td></td>
<td></td>
<td>Thermo-element</td>
</tr>
<tr>
<td>Giese 1894a</td>
<td>35.72</td>
<td></td>
<td></td>
<td></td>
<td>Thermo-element</td>
</tr>
<tr>
<td>Hertel 1900a</td>
<td>35.65</td>
<td></td>
<td></td>
<td></td>
<td>Mercury bulb</td>
</tr>
<tr>
<td>Kirisawa 1942a</td>
<td>34.50</td>
<td></td>
<td></td>
<td></td>
<td>Thermo-element</td>
</tr>
<tr>
<td>Kirisawa 1942b</td>
<td>36.34</td>
<td></td>
<td></td>
<td></td>
<td>Thermo-electric</td>
</tr>
<tr>
<td>Holmberg 1952</td>
<td>36.24</td>
<td></td>
<td></td>
<td></td>
<td>Thermo-electric</td>
</tr>
<tr>
<td>Hamano et al. 1964b</td>
<td>34.00</td>
<td></td>
<td></td>
<td></td>
<td>Thermister</td>
</tr>
<tr>
<td>Hill &amp; Leighton 1965b</td>
<td>32.10 ± 0.90</td>
<td></td>
<td></td>
<td></td>
<td>Thermistor</td>
</tr>
<tr>
<td>Mapstone 1968b</td>
<td>34.80 ± 0.30</td>
<td>33.20 - 34.80</td>
<td></td>
<td></td>
<td>Infrared</td>
</tr>
<tr>
<td>Kolstrad 1970b</td>
<td>32.00</td>
<td></td>
<td></td>
<td></td>
<td>Thermistor</td>
</tr>
<tr>
<td>Kinn &amp; Tell 1973b</td>
<td>35.00 – 36.00</td>
<td></td>
<td></td>
<td></td>
<td>Liquid crystal</td>
</tr>
<tr>
<td>Rysä &amp; Sarvaranta 1974b</td>
<td>34.80 ± 0.50</td>
<td></td>
<td></td>
<td></td>
<td>Infrared</td>
</tr>
<tr>
<td>Hørven 1975b</td>
<td>33.67</td>
<td>32.00 – 34.90</td>
<td></td>
<td></td>
<td>Contact probe</td>
</tr>
<tr>
<td>Hamano et al. 1976b</td>
<td>34.40</td>
<td></td>
<td></td>
<td></td>
<td>Infrared</td>
</tr>
<tr>
<td>Fatt &amp; Chaston 1980b</td>
<td>33.00 – 36.00</td>
<td></td>
<td></td>
<td></td>
<td>Infrared</td>
</tr>
<tr>
<td>Aliò &amp; Padron 1981b</td>
<td>32.90 ± 0.62</td>
<td>32.00 – 34.50</td>
<td>0.60</td>
<td></td>
<td>Infrared</td>
</tr>
<tr>
<td>Martin &amp; Fatt 1986b</td>
<td>34.50 ± 1.00</td>
<td></td>
<td></td>
<td></td>
<td>Heat flow</td>
</tr>
<tr>
<td>Efron et al. 1989b</td>
<td>34.30</td>
<td>32.80 – 34.50</td>
<td>0.45</td>
<td></td>
<td>Infrared</td>
</tr>
<tr>
<td>Morgan et al. 1993b</td>
<td>34.39 ± 0.47</td>
<td>32.70 – 35.40</td>
<td>0.20 ± 0.15</td>
<td>0.23 – 0.43</td>
<td>Infrared</td>
</tr>
<tr>
<td>Tan et al. 2009c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aCited by Holmberg (1952)
bCited by Tan et al. (2009c)
Before Mapstone (1968a) revolutionised OST measurement using infrared (IR) technology, the early years of labours surrounding ocular temperature measurement used the contact techniques. These included methods such as using a mercury thermometer (Holmberg, 1952), thermistors (Hill & Leighton, 1965a, Hill & Leighton, 1965b; Schwartz, 1965) and thermocouples (Rosenbluth & Fatt, 1977). Such techniques required the use of anaesthetic and the fact that it was a contact technique suggested that the results were not as accurate as heat transferred to the avascular cornea by means of conduction from the probe to the ocular surface. The contact technique also suffered from relatively poor resolution and could not be obtained instantaneously (Kinn and Tell, 1973).

![Image of bolometer and amplifier](image)

**Figure 1.5.** The bolometer (left) as well as the amplifier and recording dial (right) used to measure corneal temperature (adapted from Mapstone, 1968a).

The application of IR thermometry was pioneered by (Mapstone, 1968a). This technique involves measuring the IR energy emitted by the cornea and equating this to the relationship between IR radiance and temperature for a blackbody. IR thermometry has the major advantage of being non-contact and allowing virtually instantaneous measurement of temperature. Mapstone (1968a) started by using a bolometer (Fig. 1.5) with an IR sensitivity of 1 to 25 μm to measure the corneal temperature. The bolometer was essentially an air thermistor which produced a change in resistance when exposed to IR radiation from the ocular surface. The change was then amplified and converted by a dial into the corresponding temperature readings.

Wide-field IR thermography enables the mapping of temperature across an extended surface and has recently been applied to the study of ocular temperature. However, precise details of the variation in temperature across the ocular surface have not been presented until late 1970s (Mapstone, 1968a, Mapstone, 1968b, Mapstone, 1968c, Mapstone, 1968d, Mapstone, 1969, Mapstone, 1970). Mapstone’s works had established three important points that forms the basic principles of IR ocular thermography: Firstly, the emissivity of the cornea is between 0.97 and 1.00.
Secondly, all wavelengths greater than 3 μm emitted by the posterior ocular tissues has minimal effect on the overall OST since they are absorbed by the anterior lens, cornea and tear film. And, last but not least, the cornea can be regarded as a black body radiator since it does not transmit infrared radiation more than 2.3 μm and is assumed to be an efficient radiator in this portion of the electromagnetic spectrum.

For centuries, the interest in the temperature of the eye had never worn down. Researchers had continued to strive for better and more accurate techniques in measuring the temperature of the ocular surface which was deemed as important in many aspects.

### 1.4.3 Clinical Applications of Infrared Ocular Thermography

Efron et al. (1989) reported that Infrared (IR) ocular thermography has the advantages of instantaneous measurement of temperature. IR ocular thermography has been of great interest in recent studies of the eyes (Betney et al., 1997, Maldonado-Codina et al., 2001, Morgan et al., 1999, Purslow et al., 2005, Sodi et al., 2007, Sodi et al., 2009, Sodi et al., 2014) due to the non-invasive and more patient-friendly nature, the flexibility to measure temperature over an area rather than at a single point and the accuracy that it can produce with its passive nature (Purslow and Wolffsohn, 2005).

Clinical applications of IR ocular thermography may include physiological modeling, studying environmental influences on the temperature of the eye, monitoring corneal wound healing and detecting early ocular inflammation and dry eye as previously mentioned. Besides that, IR ocular thermography can also be used to study carotid artery stenosis (CAS) (Morgan et al., 1999), central retinal vein occlusion (CRVO) (Sodi et al., 2007), diabetic retinopathy (Sodi et al., 2009); modeling the effects of contact lens wear (Purslow et al., 2005) and patients who had undergone photorefractive keratectomy (Betney et al., 1997, Maldonado-Codina et al., 2001) as well as on age-related macular degeneration (Sodi et al., 2014).

### 1.4.4 Ocular Thermography and Tear Film

Many have suggested that the measured temperature was actually that of the tears that is spread across the cornea. When the tear film is absent, only then the radiation of the cornea can be detected. This is due to the efficient infra-red absorption / emission characteristics of the tear film, similar to water. Water is found to have an emissivity of 1, and it behaves like blackbody radiator on IR spectral above 3 μm. As tear film is close to water, the emissivity was assumed to be at 0.98 (Mapstone, 1968d) for OST measurement. Tear film is a dynamic structure, and changes in its thickness, composition and evaporation rate alter the temperature measured. In view of the current dispute over tear film thickness, it is reasonable to predict that as the tear film decreases, the cornea will have increasing influence on the radiated temperature that is measured (Craig et al., 2000).
Ocular thermography essentially assessed the tear film. Tear film had been claimed to be about 40 µm and therefore all radiation which reaches the infrared detector emanate from the tear film (Prydal et al., 1992). Thus, capturing the OST changes would reflect the changes on the tear film. As the tear film changed rapidly, it was therefore important to capture it real time using high resolution thermo-tracer that could provide real time video images with high frequency and with high sensitivity. A study done by (Purslow and Wolffsohn, 2007) has further verified that OST measurement was mainly related to tear film stability, rather than other parameters such as central corneal thickness, corneal curvature or depth of anterior chamber. The viewing angle on the curve anterior eye surface beyond 90° during ocular thermography will lead to a reduction in measured OST of 4°C or more, due to the variation of emissivity at different angle of viewing. It is therefore assumed that, the viewing angle on any part of the anterior eye during measurement is within π/4 so that the error induced due to the variation of emissivity is negligible. In such case, the OST measured can be comfortably taken as temperature of tear film even after considerations of error incurred by viewing angle and reflected thermal radiation (Tan et al., 2009a, Tan et al., 2009b).

1.4.5 Ocular Thermography in healthy eyes
Efron et al. (1989) was the first to describe the variation in temperature across the ocular surface using infrared (IR) ocular thermography (Thermo Tracer NEC6T61; NEC San-ei Instruments, Tokyo, Japan) in healthy eyes. The horizontal temperature profile and temporal stability of central cornea was established. It was well documented in the study that following a blink, the geometric centre of the cornea (GCC) had a mean temperature of 34.3 °C (range 32.8 - 35.4 °C) (Efron et al., 1989). Temperature increased towards the periphery of the cornea with the limbus being 0.45 °C warmer than the GCC and the warmest areas are at the conjunctiva due to its vasculature and increased metabolic activity. The GCC cooled at a mean rate of -0.033 °C/s over the first 15s following a blink and the magnitude of temperature change following a blink was generally less than 1 °C. The cooling rate could reflect tear stability or the ability to avoid blinking. It was postulated that measurement of corneal cooling rate and time to maintain eye opening between blink could be important tests for tear function (Efron et al., 1989). Morgan et al. (1993) reported that 95% of the normal population has an interocular temperature difference of 0.60 °C or less. Analysis of a typical thermogram reveals that the coolest part of the ocular surface is the middle of the cornea (due to its avascular nature) slightly inferior to the geometrical centre (due to coverage to the superior cornea by the upper eye lid). The isotherms are elliptical in shape due to the position of the lid margins which is an important heat source.

1.4.6 Ocular Thermography in dry eye: The Main Findings
It is worthwhile to study dry eye using ocular thermography although it could be complicated due to its multifactorial nature. Morgan et al. (1993 ) was the first to study temperature difference between limbus and central cornea (termed as RTD, radial temperature difference) on a 66 year old chronic dry eye patient and reported a significant difference in RTD between dry eye (1.40 °C) and healthy
eye (0.37 °C). Higher RTD in dry eyes was also reported to be due to faster rate of tear film evaporation (Rolando et al., 1983, Mathers et al., 1993).

Morgan et al. (1993)’s study had stimulated a great interest into measuring OST in dry eye. Many researches have been done in the area since then and the findings are summarised in Table 1.6a-c. In majority of the studies reported, dry eye was found to have the following clinical presentations as compared to controls:

(i) Dry eye has warmer OST (presented as MOST) (Morgan et al., 1995, 1996).
(ii) Dry eye has greater temperature variation across ocular surface/anterior eye (presented as radial temperature difference (RTD) (Morgan et al., 1993, 1995); temperature variation factor (TVF) (Craig et al., 2000) and compactness value (CV) (Su et al., 2011) that could be due to reduced tear film stability in dry eye.
(iii) Dry eye has faster OST cooling rate (or higher temperature difference value, TDV) and it could be attributed by higher tear evaporation rate (Morgan et al., 1996, Mori et al., 1997, Craig et al., 2000, Chiang et al., 2006, Su et al., 2011). The cooling rate was most prominent at geometrical centre cornea (GCC), followed by conjunctiva nasal (CN) and conjunctiva temporal (CT) and well correlated with TBUT (Kamao et al., 2011).
(iv) Dry eye has slower OST cooling rate and their mean corneal temperature was either lower or no difference with healthy eye (Fujishima et al., 1996, Zelichoska et al., 2005).
(v) Measuring OST using closed chamber remote sensor thermometry was superior to IR thermometry in diagnosing dry eye (Singh and Bhinder, 2005).
(vi) Measuring the change in temperature at GCC over 10 s could be diagnostic for dry eye (Kamao et al., 2011).
(vii) Measuring the CV and TDV over 6 s could be diagnostic for dry eye (Su et al., 2011).

On the other hand, Chiang et al. (2006) claimed to successfully diagnose dry eye using dynamic IR imaging with a sensitivity of 79% and a specificity of 75%, using central cornea as diagnostic index. In 2011, Kamao and his associates reported the sensitivity and specificity of TOMEY IR thermographer for the 10 seconds of sustained eye opening were 0.83 and 0.80 respectively using GCC as diagnostic index. However, the cutoff values and thermography indices studied were unclear.

Koçak et al. (1999) studied inter-occasion repeatability of ocular thermography using a noncontact infrared thermometer (THI-500) on a group of 10 healthy subjects aged 25-47 years old. Reproducibility of corneal temperature measurements was done within 45 min and within 5 days. 5 consecutive temperature readings were obtained on 1 randomly selected eye. In addition, the interocular difference in corneal temperature and the diurnal temperature changes of the cornea were assessed. Results showed that the reliability of 5 consecutive corneal temperature measurements
obtained within 45 min and within 5 days was 97.92 % and 85.35 %, respectively. It was concluded that the assessment of corneal temperature by means of non-contact infrared thermometry was highly reproducible. In 2012, Klamann and his associates further validated the inter-occasion repeatability of ocular thermography using a new ocular thermographer (Tomey TG 1000) on healthy eyes (Klamann et al., 2012). In their study, 60 eyes of 30 healthy subjects were measured 3 times over 10 seconds / session by a single examiner.

1.4.7 Ocular Thermography in dry eye: The Mechanisms

According to the literatures, mechanisms of the main findings stated in section 1.4.5 can be summarised as below:-

1. **Dry eye had warmer OST**

This was explained to be due to increased degree of conjunctival hyperaemia in dry eye (Golding, 1992, Morgan et al. (1995). An increase in ocular temperature during inflammatory disease (Mapstone, 1968b, Morgan et al., 1993) and in association with bulbar conjunctival hyperaemia (Efron et al., 1988) has been reported previously. On the other hand, it was also claimed to be due to higher blink rate in dry eye (Tan et al., 2009b).

2. **Dry eye had greater temperature variation across ocular surface / anterior eye**

There were two hypotheses given. The absence of the tear lipid layer (as the main insulating layer) in the dry eye patient is calculated mathematically as causing up to a 10-fold increase in evaporation (Scott, 1988). This resulted in cooling of the eye; heat which would have been retained by the tear layer and conducted to the cornea is lost, causing a greater temperature differential across the cornea. The second hypothesis assumes that the anterior eye with its tear film removed, the difference in temperature between central cornea and limbus will be at its maximum as there will be no lateral heat transfer by the tear film. In contrast, the temperature pattern at the surface of extraordinarily thick tear film (if there is any) would be very uniform because of heat conduction within the tear film (Morgan et al., 1993). It can thus be seen that a thicker precorneal tear film will display a smaller variation in temperature across its surface, and a dry eye with a reduced tear volume would demonstrate a much greater variation in temperature across the corneal surface.

It was reported by Rolando et al. (1983) and Mathers et al. (1993) that the evaporation rate of tears increases in dry eye and causing higher RTD. In dry eye, the absence of lipid layer resulted in an increase in the evaporation rate or aqueous deficiency would cause heat lost, resulting in a greater temperature difference across the cornea. It may also be assumed that with the absence of the tear film, being a good absorber and radiator of heat due to its high water content, there will be no lateral heat transfer by the tear film. Therefore, the difference between the central cornea and the limbus will be at its maximum, due to the difference in vasculature between these sites. It was also
found that elder individuals (age above 35) have faster rate of tear evaporation as compared to the younger individuals (55.82 Wm\(^{-2}\) and 58.9 Wm\(^{-2}\) respectively), calculated based on the first law of thermodynamics, using a sequence of thermographic images. The variation in evaporation rate was related to rate of blinking (Tan et al., 2010).

3. **Dry eye had faster OST cooling rate**

The Newton's law of cooling, indicates that the rate of heat flow between the two bodies is proportional to the difference in temperature between the two bodies. Therefore the greater rate of cooling in dry eyes can be partially explained by the observation that the OST was greater in dry eyes on eye opening. Secondly, the greater rate of cooling in dry eyes could also be attributed to a greater rate of evaporation. Two groups of workers have found between a two-fold (Rolando et al., 1983) and a three-fold (Mathers et al., 1993) increase in the evaporation rate of the tear film in dry eyes. For example, Rolando et al. (1983) measured the evaporation rate in normal subjects to be 15 g m\(^{-2}\) h\(^{-1}\) compared with 28 g m\(^{-2}\) h\(^{-1}\) in dry eyes. As cited in Morgan et al. (1996), the energy lost by the ocular surface can be calculated by multiplying these quantities by the latent heat of evaporation of water, 2260 J g\(^{-1}\); this gives the energy lost for the two groups as 9.4W m\(^{-2}\) and 17.5W m\(^{-2}\), respectively. Scott (1988) calculated the temperature distribution within the globe using finite element analysis and shown that the temperature of the ocular surface was reduced by 0.24 °C for each 20W m\(^{-2}\) of evaporation. This would suggest a steady-state reduction of 0.10 °C in eye temperature for the dry eyes described in Rolando et al. (1983)'s study. Unfortunately, the model of Scott (1988) did not address the effect of hyperaemia which will cause a higher ocular surface temperature in dry eyes as compared to normal, as shown by Morgan et al. (1995). It is evident that the calculation of Scott (1988) serve to confirm the relationship between an increased rate of evaporation and faster ocular surface cooling.
<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects (mean age)</th>
<th>Dry eye selection criterion</th>
<th>Methods</th>
<th>Results</th>
<th>Conclusion/Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morgan (1993)</td>
<td>1 D (66 yo)</td>
<td>-</td>
<td>IR thermographer NEC6T62&lt;br&gt;Sensitivity 0.1 °C&lt;br&gt;Freq: 1 frame/s&lt;br&gt;Resolution: 10x10 pixels</td>
<td>RTD higher in D&lt;br&gt;1.40 °C in D&lt;br&gt;0.37 °C in C</td>
<td>First to study RTD on dry eye</td>
</tr>
<tr>
<td>Morgan et al. (1995)</td>
<td>36 D (58yo)&lt;br&gt;27 C (57yo)</td>
<td>Require tear replacement therapy&lt;br&gt;TBUT ≤ 10 s&lt;br&gt;Schirmer &lt; 10 mm/5 min</td>
<td>IR thermographer NEC6T62&lt;br&gt;Sensitivity 0.1 °C&lt;br&gt;Freq: 1 frame/s&lt;br&gt;Resolution: 10x10 pixels</td>
<td>MOST higher in D&lt;br&gt;32.38 °C in D&lt;br&gt;31.94 °C in C&lt;br&gt;RTD higher in D&lt;br&gt;0.64 °C in D&lt;br&gt;0.41 °C in C</td>
<td>Dry eye has (i) warmer OST and (ii) greater temperature variation and correlated with shorter TBUT and poorer Schirmer results</td>
</tr>
<tr>
<td>Fujishima et al. (1996)</td>
<td>20 D (37.9yo)&lt;br&gt;20 C (35.1yo)</td>
<td>Presence of chronic dry eye symptom and at least one of the following:-&lt;br&gt;Positive results on vital staining using rose bengal and fluorescein&lt;br&gt;Abnormal tear dynamics as determined by Schirmer/TBUT/cotton thread test / tear clearance test</td>
<td>IR radiation thermometer THI-500&lt;br&gt;Sensitivity: 0.1 °C&lt;br&gt;Freq: 1 frame/s</td>
<td>Central cornea&lt;br&gt;No diff. in mean corneal temperature in D &amp; C&lt;br&gt;34.0 °C in D&lt;br&gt;34.2 °C in C&lt;br&gt;Drop in 10 s less in D&lt;br&gt;0.21 °C in D&lt;br&gt;0.61 °C in C</td>
<td>Mean corneal temperature (represented by mean GCC) in dry eye did not correlate with shorter TBUT and poorer Schirmer results&lt;br&gt;Low resolution used may limit the sensitivity in picking up minute change in temperature</td>
</tr>
<tr>
<td>Zelichowska et al. (2005)</td>
<td>9 D&lt;br&gt;13 C</td>
<td>-</td>
<td>IR radiation thermographer</td>
<td>Central cornea&lt;br&gt;Mean corneal temperature lower in D&lt;br&gt;Drop in 15 s less in D</td>
<td></td>
</tr>
<tr>
<td>Chiang et al. (2006)</td>
<td>82 D&lt;br&gt;26 C</td>
<td>-</td>
<td>Dynamic IR imaging</td>
<td>-</td>
<td>79% sensitivity&lt;br&gt;75% specificity&lt;br&gt;Area under ROC 0.841</td>
</tr>
</tbody>
</table>

**Legend:**
- **D** – Dry eye subjects
- **C** – Control subjects
- **MOST** – Mean ocular surface temperature
- **RTD** – Radial temperature difference
- **K value** – Steepening of corneal temperature
- **GCC** – Geometrical centre cornea
- **CN** – Conjunctiva nasal
- **CT** – Conjunctiva temporal
- **TDV** – Temperature difference value (temporal variation of OST)
- **CV** – Compactness value (spatial variation of OST)
- **TVF** – Temperature variation factor

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Table 1.5a. Studies of Ocular Thermography and Dry Eye reported in the literature.
<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects (mean age)</th>
<th>Dry eye selection criterion</th>
<th>Methods</th>
<th>OST acquisition</th>
<th>Results</th>
<th>Conclusion/Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morgan et al. (1996)</td>
<td>11 D (50yo) 7 C (53yo)</td>
<td>On tear replacement therapy TBUT ≤ 10 s Schirmer &lt; 10 mm/5 min</td>
<td>IR thermographer NEC6T62 Sensitivity 0.1 °C Freq: 1 frame/s Resolution: 10x10 pixels</td>
<td>Five 10x10 pixel placed in 5 anatomical locations along horizontal meridian running across centre cornea</td>
<td>Mean GCC change 1.04 °C in D 0.87 °C in C Drop in 7 s higher in D 0.88 in D 0.21 in C Cooling rate 4x higher in D -0.125 °C/s in D -0.030 °C/s in C</td>
<td>Dry eye has (i) warmer OST (ii) greater temperature variation (iii) faster cooling rate that could be due to faster tear evaporation rate</td>
</tr>
<tr>
<td>Mori et al. (1997)</td>
<td>13 D (45.5yo) 7 C (33.6yo)</td>
<td>At least one of the following:- Positive results on vital staining using rose bengal and fluorescein Abnormal tear dynamics as determined by Schirmer/TBUT/cotton thread test / tear clearance test</td>
<td>Thermal Vision Laird 3 (Nikon) Sensitivity: 0.15 °C Freq: 60 frames/s</td>
<td>20 x 20 pixel box at central cornea</td>
<td>K value decreased significantly under normal blinking in D K value decreased more under sustain eye opening a/c to normal blinking in D &amp; C</td>
<td>Blink mechanism affects tear film Relationship between corneal temperature and blink rate is unclear K value may reflect tear film stability</td>
</tr>
<tr>
<td>Craig et al. (2000)</td>
<td>8 D (60.3yo) 13 C (24.8yo)</td>
<td>NIBUT &lt; 20 s and two or more recorded dry eye symptoms with McMonnies DEQ</td>
<td>IR thermographer NEC6T62 Sensitivity 0.1 °C Freq: 1 frame/s Resolution: 10x10 pixels</td>
<td>Mean of central cornea pixels</td>
<td>Mean central corneal temperature (CCT) lower in D 33.24 °C in D 33.82 °C in C Mean temperature variation factor (TVF) higher in D 0.24 °C in D 0.17 °C in C Mean tear evaporation rate higher in D 1.48 g/m²/h in D 0.07 g/m²/h in C</td>
<td>A significant linear relationship between tear evaporation rate and temperature variation factor (TVF) established Higher latent heat of vaporisation associated with increased evaporation rate may account for faster cooling rate in dry eye</td>
</tr>
</tbody>
</table>
**Table 1.5c. Studies of Ocular Thermography and Dry Eye reported in the literature (cont.).**

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects (mean age)</th>
<th>Dry eye selection criterion</th>
<th>Methods</th>
<th>OST acquisition</th>
<th>Results</th>
<th>Conclusion/Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh and Bhinder (2005)</td>
<td>51 D (35.36yo)</td>
<td>TBUT &lt; 10 s Schirmer &lt; 10 mm/5 min Positive results on vital stain by lissamine green (&gt; 2)</td>
<td>IR and remote heat sensor thermometry</td>
<td>Closed and open eye temperature for 5 s</td>
<td>Diff in temp between closed and opened eye higher in D using IR thermometry 0.45 °C in D 0.25 °C in C Diff in temp between closed and opened eye was minor using remote sensor thermometry 0.00 °C in D 0.10 °C in C</td>
<td>Closed chamber remote sensor thermometry was superior to IR thermometry in clinching the diagnosis for dry eye as it showed no change in temperature from closed to open eye in dry eye as compared to 0.1 °C increase in normal eyes</td>
</tr>
<tr>
<td></td>
<td>51 C (35.36yo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Kamao et al. (2011)</td>
<td>30 D (52.9yo)</td>
<td>Dry eye symptom TBUT &lt; 5 s Schirmer &lt; 5 mm/5 min Positive results on vital staining by fluorescein, rose bengal or lissamine green (&gt;3)</td>
<td>Tomey IR thermographer Sensitivity 0.1 °C Freq: 6 frame/s Resolution: 320 x 240 pixels Can display results in 10 s</td>
<td>Central corneal (GCC) 4 mm in diameter, CT and CN (both 2 mm in diameter) MOST at GCC and CN was significantly different in both D &amp; C Mean drop (net change in temperature) in 10 s higher in D For GCC -0.32 °C in D -0.06 °C in C For CN -0.18 °C in D -0.01 °C in C For CT -0.17 °C in D 0.03 °C in C</td>
<td>Mean drop in 10 s most prominent at GCC, followed by CN and CT (&gt; 0.13 °C drop in GCC) and well correlated with TBUT Measuring the change in temperature at GCC over 10 s could be diagnostic for dry eye</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 C (42.7yo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Su et al. (2011)</td>
<td>76 D (49yo)</td>
<td>TBUT &lt; 10 s Schirmer with anaesthesia &lt; 5 mm/5 min</td>
<td>Microbolometer sensor Sensitivity 0.1 °C Freq: 30 frame/s Resolution: 320 x 240 pixels ROI (region of interest) determined by four curves connected between four manually set apexes ( top and bottom of the eye, left and right corner of the eye)</td>
<td>Mean TDV in 6 s higher in D 0.75 °C in D 0.48 °C in C Mean CV in 6 s (of relative-lower-temp-area) higher in D 31.5 °C in D 16.1 °C in C</td>
<td>Measuring TDV and CV could be diagnostic for dry eye Higher TDV may be attributed to faster evaporation rate in dry eye Higher CV indicates tear instability in dry eye Recommend 6 s measurement time as it is more comfortable for patient and reduce reflex tearing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47 C (34yo)</td>
<td></td>
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</table>
1.5 Summary

It is evident that ocular thermography has the capability to assess tear film and can be a novel tool to assess and diagnose dry eye. Various efforts have been made to prove that and the results were however rather scattered. Furthermore, OST indices that could substantially diagnose dry eye remain unclear. Most studies were focusing on 1-3 indices include: the geometric center of the cornea (GCC) (Efron et al., 1989, Morgan et al., 1996, Craig et al., 2000, Kamao et al., 2011, Koçak et al., 1999), radial temperature differences (RTD) (Morgan et al., 1993, Morgan et al., 1995), mean ocular surface temperature (MOST) (Morgan et al., 1995, Singh and Bhinder, 2005a), conjunctiva nasal and temporal (CN and CT) (Kamao et al., 2011), minimum and maximum temperature of the anterior eye (Klamann et al., 2012). In this study, twelve OST indices were included to study the whole of the exposed ocular surface (within region of interest, ROI). A couple of new OST indices have been included: temperature standard deviation (SD) of the ROI and temperatures of the nasal and temporal limbus (LN and LT).

Fujishima et al. (1996) has reported that the resolution of the thermometer used were too low and may limit the sensitivity in picking up minute change in OST. The thermo-tracer that previously used (Morgan et al., 1995, Morgan et al., 1996) was one which recorded still images with low operational sensitivity of 0.1 °C and at a low capturing rate of 1.0 frame/s with low resolution (10 x 10 pixels). With modern technology, thermos-tracer with high resolution and added features had been made available. The NEC Thermo Tracer TH 9260 (NEC Avio Technologies Co., Ltd., Tokyo, Japan) used in this study had a higher resolution (640 x 480 pixels), operational sensitivity (0.06 °C) and frequency (30 frames per second) making it worth repeating Morgan’s study on a larger sample size as proposed by Morgan et al. (1996) and exploring new findings. The thermos-tracer was also capable of capturing real time video images so that the dynamic tear film pattern would be analysed more accurately.

Ocular surface marking and OST acquisition used in the literature were shown to be inconsistent. The current study proposed a new method of cropping and marking the ocular surface and investigated the repeatability of OST acquisition. The variability of palpebral aperture size which could be very prominent in Asian eyes due to their relatively smaller aperture size as compared to the Caucasian eyes was taken into account. While dynamic ocular thermography has been reported to have high accuracy and sensitivity (Tan et al., 2009c) but little has been reported about its repeatability. Koçak et al. (1999) and Klamann et al. (2012) studied inter-occasion repeatability on healthy eyes only. In the current study, the repeatability of NEC Thermo Tracer TH 9260 in three aspects (inter-image, inter-occasion and inter-examiner) was evaluated in both healthy and dry eyes.
1.6 Objectives of the thesis

1. To investigate the prevalence of dry eye in Singapore population.
2. To study inter-image, inter-occasion and inter-examiner repeatability of ocular thermography in assessing healthy and dry eyes.
3. To evaluate the ability of ocular thermography in assessing the tear film and potentially as a new diagnostic tool for dry eye. The important temperature metrics in diagnosing dry eye were derived.
4. To compare the novel approach with conventional methods of assessing dry eye.

1.7 References


Johnson ME and Murphy PJ. Temporal changes in the tear menisci following a blink. Experimental Eye Research 2006; 83(3): 517-525.


Kallarackal GU, Ansari EA, Amos N, Martin JC, Lane C, Camilleri JP. A comparative study to assess the clinical use of Fluorescein Meniscus Time (FMT) with Tear Break up Time (TBUT) and Schirmer’s tests (ST) in the diagnosis of dry eyes. Eye 2002; 16(5): 594-600.


Val and Rocular surface temperature. Purslow C


2 Methods and Preliminary results

2.1 Ocular thermography apparatus and its specifications

Infrared (IR) ocular thermography is a non-contact technique for measuring the ocular surface temperature (OST) using an IR detector which is linked to a computer terminal that allows user control via a compatible software program. An IR sensitive unit inside the detector unit absorbs radiation emitted by the ocular surface and converts the measurements into temperature readings (Fig 2.1). The results are displayed in a 2-dimensional thermogram which shows the distribution of the temperature over the ocular surface in gray scale or red-green-blue (RGB) colour palette (Fig. 2.2). An elliptical corneal isotherm is typically revealed on an ocular thermogram. The horizontal axis of the isotherm is longer due to the shape of the palpebral aperture (Efron et al., 1989, Morgan et al., 1993).

Figure 2.1. Schematic drawing of ocular thermographer in the clinic room (adapted from Morgan et al., 1993)

Figure 2.2. Thermogram of the ocular surface in gray scale (left) and RGB palette (right).
Being a non-contact method, IR ocular thermography has several advantages. Firstly, it does not cause any discomfort or trauma to the subjects. Hence, there is no need for any form of anaesthesia. Next, this technique measures the IR radiation emitted by the ocular surface so it does not alter nor interfere with the temperature being measured. Furthermore, the temperature distribution of the entire ocular surface can be displayed in real time. The NEC IR thermo tracer TH 9260 (NEC Avio Technologies Co., Ltd., Tokyo, Japan) used in this study is shown in Fig. 2.3 and the patient-examiner set up is shown in Fig. 2.4.

Figure 2.3. NEC infrared thermo tracer TH 9260 used in the study.

Figure 2.4. Patient-examiner set up for ocular thermography
Changes in OST after a blink can be very fast and minute (Efron et al., 1989, Morgan et al., 1996). Hence, the ability to measure and record the changes in OST over time is invaluable for the study of post-blink temperature changes. To capture the dynamic changes in OST accurately, IR detectors with high sensitivity and speed are required; that used in this study has a thermal resolution of 0.06 °C and a frame time of 30 frames per second. It is a huge improvement over the older models that had an optimal operational sensitivity of 0.1 °C and could only capture 1 fps. The specifications of NEC IR thermo tracer TH 9260 (NEC Avio Technologies Co., Ltd., Tokyo, Japan) is shown in Table 2.1.

Table 2.1. Specifications of NEC infrared thermo tracer TH 9260.

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>640 (H) x 480 (V) pixels</td>
</tr>
<tr>
<td>Operational sensitivity</td>
<td>0.06 °C</td>
</tr>
<tr>
<td>Frequency</td>
<td>30 frames/second</td>
</tr>
<tr>
<td>Range of IR radiation</td>
<td>8 and 14 µm</td>
</tr>
<tr>
<td>Emissivity*</td>
<td>0.98</td>
</tr>
</tbody>
</table>

*Mapstone, 1968d

2.2 The engineering and computational aspects of infrared ocular thermography

The technological advances in infrared (IR) sensor technology and image processing make it possible to use IR detector to capture OST and analyse the OST images. The devices provide an image using based on infrared radiation, similar to a visual camera that forms an image using visible light. Instead of the 450–700 nanometer (nm) range of the visible light camera, IR thermo tracer operate in wavelengths between 7 µm and 14 µm (Tan et al., 2011).

Most high end modern IR detectors are cooled to 60 K to 100 K, depending on the type and performance level. Without cooling, these infrared sensors would be flooded by their own radiation. Cooling requires sophisticated electronics meaning that IR detectors are expensive both to produce and the device may need several minutes to cool before it can begin working. The uncooled IR detectors use a sensor operating at or close to ambient temperature using small temperature control elements. Modern uncooled detectors use sensors that work by the change of resistance, voltage or current when heated by infrared radiation. These changes are then measured and compared to the values at the operating temperature of the sensor. Uncooled infrared sensors can be stabilized to an operating temperature to reduce image noise, but they are not cooled to low temperatures and do not require bulky, expensive cryogenic coolers. This makes the device smaller and less costly but relatively lower resolution and image quality compared to cooled detectors. Current improvements of uncooled focal plane arrays are primarily on higher sensitivity and pixel density. For IR ocular thermography, the uncooled IR detectors are commonly used (Tan et al., 2011).
Currently, thermal imaging is being used for OST studies in normal and diseased eyes (Efron et al., 1989, Morgan et al., 1993, Tan et al., 2009, Tan et al., 2011). Three important aspects need to be addressed. They are: capturing high quality OST images, selecting image features and how to cluster and classify OST images.

2.2.1 Capturing high quality OST images
To capture high quality OST images, the following components regarding the IR thermo tracer need to be taken into account: (1) dynamic range, (2) resolution and sensitivity; (3) IR thermo tracer calibration; (4) optical focus, thermal focus, and palette choice.

2.2.1.1 Dynamic range
Dynamic range refers to the ability of an IR thermo tracer to preserve fine temperature details in the presence of large scene temperature range. It is determined by the thermo tracer’s image digitisation and formation electronics. Each gray tone or colour on the image represents a specific electrical impulse level, which corresponds to the thermal energy levels in the image, and falls within a range of temperature. The range feature allows for the selection of a section of infrared energy levels. This temperature range must encompass the specific temperatures being imaged. The range of “energy levels” is the first thing to consider when thermally focusing the object of interest. The full dynamic range on most IR thermo tracers is 12-bit and therefore capable of outputting 4096 electrical impulse levels. The electrical impulses are displayed as a 256-color (8-bit) pallet (Wu et al., 2006) (Fig. 2.5).

![Dynamic range of 12 bits](image)

Figure 2.5. Dynamic range of 12 bits.

2.2.1.2 Resolution and sensitivity
Resolution and sensitivity are two of the most important parameters for an IR thermo tracer. The resolution can be divided into temperature resolution and spatial resolution. The temperature resolution is analogous to the number of colours in a computer display. The better the temperature resolution, the smoother will be the temperature transitions. The typical temperature resolution for high-end IR thermo tracer that is used in OST study is 0.03 °C – 0.06 °C.
The spatial resolution is determined by the size of the pixel count. The higher the spatial resolution, the sharper is the image. In general, an IR thermo tracer with 320 x 240 pixels is adequate for study of OST due to the small area of eye. However, the images may appear to be grainy if these images are magnified. Larger pixel counts such as 640 x 480 pixels will ensure that the images will contain more useful thermal details (Razeghi, 1998).

The thermal sensitivity is measured in degree Celsius (°C), which determines the minimum temperature difference that can be detected. The sensitivity is an extremely important factor when selecting the IR detectors. The lower the sensitivity, the more accurate the detectors can be as well as producing more detailed images. Highly sensitive IR detectors will show more colour / temperature differences. Thermal sensitivity has a direct correlation with the accuracy of the IR thermo tracer as well (Razeghi, 1998). The thermo tracer TH9260 used in this study has a thermal sensitivity of ± 2°C.

2.2.1.3 Colour palette, Optical and Thermal focus

To have a good thermal image for analysis, the optical focus, thermal focus and choice of colour palette are critical. As the eye is placed at a short distance to the IR thermo tracer during operation (8 cm – 10 cm), a close-up lens is required so that the focus can be done and proper size of OST images can be captured. For the choice of colour palette, it is advised to always begin with the gray palette to optically focus the image as the grayscale pattern is similar to a normal visual scene. This can then be changed to the colour palette of choice and “thermally focus” the image. Thermal focusing involves the adjustment of ‘span’ and ‘level’. In the case of the current apparatus, this can be set on the thermo tracer unit or using the software bundled with the IR thermo tracer after the IR image has been saved. ‘Span’ (also called gain, thermal contrast or sensitivity) is defined as a temperature range around the median point of the scale (Corsi, 2010). It is an adjustable “window” which selects a range of thermal energy levels observable within the full measurement range of the thermo tracer. When a small span of thermal levels is displayed, the thermal image contrast increases. This means more temperature details can be seen (Kozlowski and Kosonocky, 1995). A larger span decreases the contrast (Fig. 2.6). Setting a narrower span allows better resolution of the images and higher accuracy in the measured temperatures. For that reason, images will better illustrate smaller temperature differences. However, different brands of IR thermo tracers may have different minimum span. On the other hand, a broader scale and higher maximum temperature range may be needed to prevent saturation of the portion of the image at the highest temperature.

‘Level’ (also called thermal brightness) is the control that allows the operator to adjust where on Full Range to move the Span. A small Level gives a brighter image and a large Level gives a darker image (Fig. 2.7). It can be compared to visual brightness on a video thermo tracer (Corsi, 2010).
Figure 2.6. More temperature details can be seen with smaller span: (a) small span, (b) large span.

Figure 2.7. Thermal image is brighter with smaller level: (a) small level, (b) large level.

2.2.2 Selecting image features

There are different methods that describe the features of OST. One group of methods is based on the calculation of statistical parameters for the region of interest (ROI). These parameters such as minimum temperature, maximum temperature, average temperature (m), standard deviation (σ), skewness, and kurtosis can be used to compare and separate OST images (Razeghi, 1998). The next group of methods is based on image transformations such as linear filtering, Fourier or wavelet analysis.

The definitions of the statistical parameters are given below:

\[
m = \frac{1}{N} \sum_{k=1}^{N} f(k)
\]

\[
\sigma = \sqrt{\frac{1}{N} \sum_{k=1}^{N} (f(k) - m)^2}
\]
Skewness = \frac{1}{N\sigma^3} \sum_{k=1}^{N} (f(k) - m)^3

Kurtosis = \frac{1}{N\sigma^4} \sum_{k=1}^{N} (f(k) - m)^4

where \( f(k) \) and \( N \) represent the temperature at location \( k \) and total number of pixels in ROI respectively.

Standard deviation is a widely used measure of the variability. It shows how much variation there is from the "average" (mean). A low standard deviation indicates that the data sets tend to be very close to the average, whereas high standard deviation indicates that the data are spread out over a large range of values.

Skewness is a measure of symmetry. A distribution is symmetric if it looks the same to the left and right of the centre point. The skewness for a normal distribution is zero. Any symmetric data has skewness near zero. Negative skewness indicates data that are skewed left and positive skewness indicates data that are skewed right. Skewed left means that the left tail is long relative to the right tail and skewed right means that the right tail is long relative to the left tail.

Kurtosis is a measure of whether the data are peaked or flat relative to a normal distribution. Data sets with high kurtosis tend to have a sharp peak near the mean, decline rapidly, and have heavy tails. Data sets with low kurtosis tend to have a flat top near the mean rather than a sharp peak (Razeghi, 1998).

Wavelet transformation is used in many applications, such as signal compression and feature extraction (Najarian and Splinter, 2006). It employs low-pass and high-pass decomposition filters to decompose the input image into four lower resolution sub-bands which contain approximation (low frequency), horizontal, vertical, and diagonal detail (high frequency) coefficients respectively. New features from different sub-bands could be generated. The wavelet transformation coefficients in each sub-band are used as complexity feature. In OST study, both the changes of OST and the scale of the changes are important. In general, the wavelet coefficients in high frequencies are used since these coefficients reflect detailed high-frequency changes across the OST image. In order to better understand the use of high-frequency coefficients, assuming two OST images that are captured from a same subject at different time are compared: one image is taken when the eye is normal; the other is taken when the eye disease is found. The overall similarity of the two images indicates that the wavelet coefficients describing the approximation of the two images are very similar. It means that the wavelet coefficients in the low frequencies are very similar, but assuming that the two images are different, the wavelet coefficients describing the details of the two images must be different. This indicates that a comparison of the high-frequency coefficients should reveal the differences between the two images (Najarian and Splinter, 2006).
2.2.3 Clustering and Classification

Thermal image clustering and classification are powerful tools for OST analysis. Among the variety of different image features, statistical parameters have been effectively used for classification. In other approaches, the features obtained from wavelet transformation can also be used for successful classification. Many clustering and classification methods have been proposed, such as K-means (MacQueen, 1967), hierarchical (Fowlkes and Mallows, 1983), and fuzzy c-mean (Bezdek, 1981) for clustering; Bayesian method, Maximum likelihood method, and neural networks for classification (Najarian and Splinter, 2006).

K-means is one of the most popular clustering methods used in image analysis. It aims at minimizing an objective function, in this case a squared error function. The objective function is defined as follows:

\[ J = \sum_{i=1}^{K} \sum_{j=1}^{N} \| f_{i}^{(j)} - C_{j} \|^2 \]

where \( \| f_{i}^{(j)} - C_{j} \|^2 \) is a chosen distance measure between a data point \( f_{i}^{(j)} \) and the cluster centre \( C_{j} \), is an indicator of the distance of the N data points from their respective cluster centres (MacQueen, 1967).

K-means method consists of the following steps (MacQueen, 1967):
1. Place K points into the space represented by the objects that are being clustered. These points represent initial group centroids.
2. Assign each object to the group that has the closest centroid.
3. When all objects have been assigned, recalculate the positions of the K centroids.
4. Repeat Steps (2) and (3) until the centroids no longer move. This produces a separation of the objects into groups from which the metric to be minimized can be calculated.

Bayesian method is one of the most used classification methods (Najarian and Splinter, 2006). The concepts of the Bayesian theory was described with a simple example that deals with detecting OST images for diseased eyes. In such an application there were two classes: a class of OST images for diseased eyes and a class of OST images for normal eyes. The class of the sample with standard deviation (STD) was denoted. For the OST image of a diseased eye, \( \sigma = \sigma_1 \) and for the OST image of a normal eye, \( \sigma = \sigma_2 \).

According to the Bayesian theory,

\[ P(\sigma_i|X) = \frac{P(X|\sigma_i)P(\sigma_i)}{P(X)} \]

\( P(\sigma_i|X) \) is called the a posterior probability which quantifies the likelihood that a given OST image belongs to class \( \sigma_i \) when the standard deviation (STD) of the selected image is \( X \). \( P(X|\sigma_i) \) is a
conditional probability which quantifies the probability that the STD of an image belonging to class \( \sigma_i \) will be in the range of \( X \). \( P(\sigma_i) \) denotes a priori probability of the class \( \sigma_i \). This priori probability can be estimated if a large sample of data is available. \( P(X) \) is a normalising factor, which can be disregarded in the decision-making process since it is equal for all classes. For this simple example, the decision-making process becomes a matter of choosing \( \sigma_1 \) if \( P(X|\sigma_1)P(\sigma_1) > P(X|\sigma_2)P(\sigma_2) \) and \( \sigma_2 \) otherwise (Najarian and Splinter, 2006).

### 2.3 Calibration and repeatability study

IR thermo tracer calibration enable the device to operate to its optimum performance ensuring measurement accuracy and reliability. It is important to calibrate the thermo tracer before it is used in the measurement of OST.

The same calibration method as reported by Tan et al. (2009) was adopted. To calibrate the NEC IR thermo tracer TH 9260, a blackbody (BB702) was set at 16 individual temperatures from 32.20 to 40.60 °C. The temperature was randomly selected and masked from an examiner. Each of the surface temperature set on the BB702 blackbody was measured 5 x using NEC IR thermo tracer TH 9260 (Fig. 2.8). The readings were then averaged and plotted (Figure 2.9). A regression equation of the relationship between blackbody and thermo tracer readings was then derived.

![Figure 2.8. Set up of the blackbody (BB702) and thermo tracer at a distance of 10 cm.](image)

Figure 2.9 shows the relationship between temperature of the BB702 blackbody radiator (y-intercept) and thermo tracer readings (x-intercept). All future thermo tracer readings can be converted to ‘true’ temperature via the regression equation derived from the graph \( y = 0.9787x + 0.6508 \) (\( R^2 = 0.9950 \)), where \( y \) is the ‘true’ temperature and \( x \) is reading by thermo tracer. On the other hand, when the surface temperature of the blackbody was set at 36.1 °C (97 °F) and
measured 34 times using the thermo tracer, mean and SD was found to be $36.5 \pm 0.1 ^\circ C$. Therefore the accuracy of the machine was $+0.3$ to $+0.5 ^\circ C$. A factor of 0.4 was suggested to be subtracted to all OST measurement if the same type of thermo tracer is used in the future. On the other hand, it is found to be able to measure temperatures within a sensitivity range of $\pm 0.1$ to $0.2 ^\circ C$.

![Calibration of TH9260](image.png)

**Figure 2.9.** Temperature of the blackbody radiator (BB702) compared to that measured by thermo tracer TH9260. N = 5 repeat readings. Error bars represent 1 SD.

To study the repeatability of the NEC IR thermo tracer TH 9260, two repeated measurement were done at 20 min interval by an examiner when on BB702 blackbody was set at the same range of temperature ($32.20 - 40.60 ^\circ C$ or $90 - 105 ^\circ F$). Repeatability of the two set of measurement was calculated using the method recommended by (Bland and Altman 1986, Bland and Altman 1996). Coefficient of repeatability (COR) (BSI 1979) associated with repeated measurements at two different occasions was calculated. In brief, the COR is $2.77 \times$ the average standard deviation ($Sw$) of two sets of repeated measurement. The COR values estimates the maximum difference likely to occur between 95% of pairs of successive measurements on BB702. Results were presented in COR (%COR). In addition, paired t-tests were done. Lastly, the mean of the analysis/reanalysis OST was plotted against the difference between these two measurements using the approach suggested by Bland and Altman (1986) to explore the relationship between measurement error and measurement magnitude and to derive the 95% limits of agreement (Bland and Altman, 1986).
Table 2.2. Summary of inter-measurement repeatability of TH9260 against BB702 blackbody: the p values (paired t-test), within-measurement standard deviation (Sw), coefficient of repeatability (COR) and its percentile are shown.

| Overall mean (range) set on BB702 (°C) | 36.39 (32.20 - 40.60) |
| Overall mean (range) measured by TH9260 (°C) | 36.26 (32.00 - 40.30) |
| p values (paired t-test) | 0.98 |
| Sw | 0.10 |
| COR (%COR) | 0.34 (0.94) |

Table 2.2 shows a summary of the repeatability results. There was no significant difference between the distributions of differences for the two repeated measurements at 95% CI (paired t-test, p > 0.05). Inter-measurement COR (%COR) shows that the maximum difference likely to occur between 95% of the two successive measurements by a single examiner was found to be small COR (%COR) of 0.34 (0.94). Within-measurement standard deviation (Sw) was also shown to be small (0.10). Bland Altman plots for inter-measurement analysis/reanalysis is shown in Fig. 2.10. The 95% limits of agreement and mean difference are shown in the plots. Findings suggest that there was no consistent trend for the differences to alter with increasing mean values.

Figure 2.10. Bland Altman plot for inter-measurement analysis/reanalysis. Dotted lines on the plot show the 95% limits of agreement.

In conclusion, NEC IR thermo tracer TH 9260 (NEC Avio Technologies Co., Ltd., Tokyo, Japan) was shown to be repeatable. This was valid as the two sets of measurement have presented with small %COR at 95% CI, small average standard deviation (Sw) values in relative to their mean absolute values and there was no consistent trend for the differences to alter with increasing mean values in Bland-Altman plots at 95% CI.
2.4 Study design and protocol

2.4.1 Dry eye prevalence study

A cross-sectional dry eye survey was carried out using the McMonnies dry eye questionnaire (DEQ) at 46 (out of 62) randomly selected mass rapid transit (MRT) stations and their vicinity over the period of four months in 2010. The results of this study are presented in Chapter 3.

2.4.1.1 Study protocol

Synopsis

Short title: Prevalence of and risk factors for symptomatic dry eye disease in Singapore
Methodology: Cross-sectional, population-based
Number of participants: 1004 questionnaires
Age of subjects: 15 and 83 years old
Investigators: Li Li Tan, Zhi Qiang Cai, Philip B. Morgan
Location: Mass rapid transit (MRT) stations and their vicinity over Singapore
Planned schedule: May to August 2010

A cross-sectional dry eye survey was carried out using the McMonnies DEQ. Members of the public were interviewed at the 46 (out of 62) randomly selected mass rapid transit (MRT) stations and their vicinity. A total of 1004 questionnaires were collected for participants aged between 15 and 83 years old. Symptomatic dry eye disease (SDED) was defined as those with at least one of five self-reported symptoms that were reported as often or constantly. Non-dry eye (NDE) subjects were those with no related symptoms reported. Prevalence of SDED in the studied population and confidence interval (CI) were calculated. Risk factors were also evaluated using logistic regression analysis at 95% CI.

2.4.1.2 Objectives

To describe the prevalence and risk factors of symptomatic dry eye disease (SDED) in Singapore.

2.4.1.3 Selection of study population

Sample size calculation

This cross-sectional study was carried out May to August 2010. Before commencement, the appropriate sample size was determined as follows. Assuming a margin of error of ± 5% and (p) as the estimated prevalence of dry eye in Singapore, the sample size needed for this study was:

\[ n = \frac{p \times (1-p) \times (1.96/0.05)^2}{(95\% \text{ confidence level})} \]

Assuming a worst case scenario of \( p = 0.5 \), the minimum sample size needed for the required margin of error was 385.
Sampling method to represent Singapore population

The Mass Rapid Transport (MRT) is a common mode of transport amongst Singaporeans and is evenly distributed across the island of Singapore and use of MRT stations was chosen as a suitable way to seek out study participants. The passenger numbers for the MRT have been rising steadily and were up to about 39 million per month in 2007 (Chio, 2008).

Forty-six of the 62 MRT stations were randomly selected to be study sites and covered all parts of Singapore. Members of the public were approached at the MRT stations and in nearby locations such as shopping malls, food centres and shops in order to reach an appropriately representative sample of the Singapore population.

2.4.1.4 Ethical conduct of the study

This study was conducted in compliance with the ethical principles that have their origins in the Declaration of Helsinki (2008). Ethical approval for the study was obtained from the Singapore National Health Group (NHG) Domain-Specific Review Board (DSRB) (appendix 2.1a & b) and the Singapore Polytechnic ethics review committee. Informed consent was obtained from each subject at study enrolment.

All participant data was held strictly confidential and the research team conformed to the Data Protection Act of 1998 with respect to data collection, storage and destruction. Data was kept on a password-protected computer in a lockable room at the Singapore Polytechnic. Only the research team had access to the data. In cases where the results were published in a thesis or a scientific journal, no identifiable characteristic of the participant was disclosed (e.g. name, date of birth). The participants were free to withdraw from the study at any time (Declaration of Helsinki), without any obligations.

2.4.1.5 Patient information sheet and consent

Member of the public were approached and invited to complete the McMonnies DEQ. The participants were interviewed in either English or Mandarin. Surveyors were conversant in both languages, and the participant's responses were translated into English to ensure that there were no discrepancies. Majority of the participants could readily comprehend English. All participants were given a verbal explanation of the nature of the study along with an information sheet. The sheet explained the rationale of the study, the procedures and time involved, any potential risk or benefit to the participant, ethical considerations (e.g. confidentiality, withdrawal), and investigator contact details. Participants were then invited to participate in the second part of the study (2.4.3: The Main Study). Participants were given sufficient time (two weeks) to decide whether they wanted to participate in the second part of the study and to contact investigator once decided. A copy of the approved DSRB application form can be found at appendix 2.2 and a copy of the subject information sheet and consent form can be found at appendix 2.3.
2.4.1.6 **Statistical analysis**

The prevalence of symptomatic dry eye disease (SDED) in the studied population and confidence interval (CI) were calculated for the 15 studied risk factors. Potential risk factors were then ruled out (those with $p \leq 0.06$) and further evaluated using logistic regression analysis to rule out the ‘true’ risk factors at 95% CI.

2.4.2 **Inter-image, inter-occasional and inter-examiner repeatability of infrared ocular thermography in assessing healthy and dry eyes**

A single masked controlled study was carried out at the Khoo Teck Puat hospital eye clinic over the period of one year in 2011. The results of this study are presented in Chapter 4.

### 2.4.2.1 Study protocol

**Synopsis**

Short title: Repeatability of infrared ocular thermography in assessing healthy and dry eyes  
Methodology: Monocentre, single masked controlled study  
Number of participants: 21 healthy and 15 dry eye subjects  
Age of subjects: 30 to 55 years old  
Gender/ethnicity: no restriction  
Investigators: Li Li Tan, Srinivasan Sanjay, Philip B. Morgan  
Location: Khoo Teck Puat Hospital, Yishun, Singapore  
Planned schedule: Saturday, 2011, 9 am to 2 pm.

### 2.4.2.2 Objectives

To investigate the repeatability of NEC thermo tracer TH9260 (NEC Avio Technologies Co., Ltd., Tokyo, Japan) (in assessing healthy and dry eyes and to identify the most repeatable ocular temperature metrics.

### 2.4.2.3 Selection of study population

Dry eye subjects were screened and recruited by an ophthalmologist (Dr. Sanjay) based on the inclusion criterion.

Healthy/control subjects were self-recruited via internal email to Singapore Polytechnic staffs and patients visited the Singapore Polytechnic Optometry Centre.

### 2.4.2.4 Inclusion criterion

**Inclusion criterion for dry eye subjects:**

- Ability to provide written informed consent
- Male or female aged 30 to 55 years old
- Corrected far VA at least 6/9 Snellen
- Use of tear replacement therapy
Had either a fluorescein tear breakup time of 10 seconds or less, or a Schirmer I test result of less than 10 mm in 5 min (Morgan et al. 1995)  
Presence of corneal or conjunctiva staining  
Good general health / not taking medication that will affect tear film  
No contact lens for past two years

Inclusion criterion for healthy subjects/controls:-  
Male or female aged 30-55 years old  
No signs and symptoms of dry eye  
Good general and ocular health  
No contact lens use for past two years

2.4.2.5 Exclusion criterion  
Any anterior ocular anomalies (e.g. current ocular infection, allergy, ptosis)  
Any anterior eye surgery (e.g. LASIK)  
Any remarkable posterior health anomalies such as optic atrophy/neuropathy.  
Use of systemic medications known to affect tear film: e.g. medication for hypertension, diabetes mellitus etc.  
Currently pregnant or breastfeeding/hormone replacement therapy  
Smoker  
Inability of participant to understand the study procedures and provide informed consent  
Use of contact lenses regularly two years prior to study commencement.

2.4.2.6 Ethical conduct of the study  
This study was conducted in compliance with the ethical principles that have their origins in the Declaration of Helsinki (2008). Ethical approval for the study was obtained from the Singapore National Health Group (NHG) Domain-Specific Review Board (DSRB) (appendix 2.1a & b) and the Singapore Polytechnic ethics review committee. Informed consent was obtained from each subject at study enrolment.

All participant data was held strictly confidential and the research team conformed to the Data Protection Act of 1998 with respect to data collection, storage and destruction. Data was kept on a password-protected computer in a lockable room at the Singapore Polytechnic. Only the research team had access to the data. In cases where the results were published in a thesis or a scientific journal, no identifiable characteristic of the participant was disclosed (e.g. name, date of birth). The participants were free to withdraw from the study at any time (Declaration of Helsinki), without any obligations.
2.4.2.7 Patient information sheet and consent
Volunteers who contacted the investigator were given a verbal explanation of the nature of the study along with an information sheet. The sheet explained the rationale of the study, the procedures and time involved, any potential risk or benefit to the participant, ethical considerations (e.g. confidentiality, withdrawal), and investigator contact details. Volunteers were given sufficient time (two weeks) to decide whether they wanted to participate in the study. A copy of the approved DSRB application form can be found at appendix 2.2 and a copy of the subject information sheet and consent form can be found at appendix 2.3.

2.4.2.8 Study procedure and visit schedule
Study investigations were conducted as per the schedule shown in Table 2.3 below. It was a single visit lasted approximately one hour with comfort breaks. Subjects were asked to stay in the room with controlled environment for the entire procedures. A copy of the record form can be found at appendix 2.

Table 2.3. Study flow chart 1.

<table>
<thead>
<tr>
<th>Study Procedures</th>
<th>Single visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
<td>✔</td>
</tr>
<tr>
<td>Verification of inclusion and exclusion criterion</td>
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<tr>
<td>Optimal optical correction</td>
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<tr>
<td>Best far corrected VA (Snellen)</td>
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<tr>
<td>Direct ophthalmoscopy (posterior health)</td>
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<tr>
<td>Slit Lamp Biomicroscopy (anterior health)</td>
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</tr>
<tr>
<td>Infrared ocular thermography</td>
<td>✔</td>
</tr>
</tbody>
</table>

2.4.2.9 Statistical analysis
Data was tabulated and analysed using various statistical software and presented in Chapter 4.

2.4.3 The main study
A single masked controlled study was carried out at the Khoo Teck Puat hospital eye clinic over the period of two years in 2012 to 2013. The results of this study are presented in Chapter 5, 6 and 7.

2.4.3.1 Study protocol

Synopsis
Short title: Assessing and diagnosing dry eye disease (DED) using infrared ocular thermography and comparing with conventional dry eye tests
Methodology: Monocentre, single masked controlled study
Number of participants: 62 dry eye patients and 82 controls.
Age of subjects: 30 to 55 years old
Gender/ethnicity: no restriction
2.4.3.2 Objectives

(i) To investigate dry eye disease using IR ocular thermography.
(ii) To evaluate the efficacy of IR ocular thermography as a diagnostic tool for DED.
(iii) To study the diagnostic ability of various conventional dry eye tests, their correlation with ocular surface temperature and derive the best composite/combined tests for DED.

2.4.3.3 Selection of study population

Dry eye subjects were screened and recruited by an ophthalmologist (Dr. Sanjay) based on the inclusion criterion.
Healthy/control subjects were self-recruited via internal email to Singapore Polytechnic staffs and patients visited the Singapore Polytechnic Optometry Centre.

2.4.3.4 Inclusion criterion

Inclusion criterion for dry eye subjects:
- Ability to provide written informed consent
- Male or female aged 30 to 55 years old
- Corrected far VA at least 6/9 Snellen
- Use of tear replacement therapy
- Had either a fluorescein tear breakup time of 10 seconds or less, or a Schirmer I test result of less than 10 mm in 5 min (Morgan et al. 1995)
- Presence of corneal or conjunctiva staining
- Good general health / not taking medication that will affect tear film
- No contact lens for past two years

Inclusion criterion for healthy subjects/controls:
- Male or female aged 30-55 years old
- No signs and symptoms of dry eye
- Good general and ocular health
- No contact lens use for past two years

2.4.3.5 Exclusion criterion

Any anterior ocular anomalies (e.g. current ocular infection, allergy, ptosis)
Any anterior eye surgery (e.g. LASIK)
Any remarkable posterior health anomalies such as optic atrophy/neuropathy.
Use of systemic medications known to affect tear film: e.g. medication for hypertension, diabetes mellitus etc.
Currently pregnant or breastfeeding/hormone replacement therapy
Smoker
VA 6/12 Snellen or worse
Inability of participant to understand the study procedures and provide informed consent
Use of contact lenses regularly two years prior to study commencement

2.4.3.6 Ethical conduct of the study
This study was conducted in compliance with the ethical principles that have their origins in the Declaration of Helsinki (2008). Ethical approval for the study was obtained from the Singapore National Health Group (NHG) Domain-Specific Review Board (DSRB) (appendix 2.1a & b) and the Singapore Polytechnic ethics review committee. Informed consent was obtained from each subject at study enrolment.

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2.4.3.7 Patient information sheet and consent
Volunteers who contacted the investigator were given a verbal explanation of the nature of the study along with an information sheet. The sheet explained the rationale of the study, the procedures and time involved, any potential risk or benefit to the participant, ethical considerations (e.g. confidentiality, withdrawal), and investigator contact details. Volunteers were given sufficient time (two weeks) to decide whether they wanted to participate in the study. A copy of the approved DSRB application form can be found at appendix 2.2 and a copy of the subject information sheet and consent form can be found at appendix 2.3.

2.4.3.8 Study procedure and visit schedule
Study investigations were conducted as per the schedule shown in Table 2.4 below. It was a single visit lasted approximately one hour with comfort breaks. Subjects were asked to stay in the room with controlled environment for the entire procedures. A copy of the record form can be found at appendix 2.4.
Table 2.4. Study flow chart 2.

<table>
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<tr>
<td>Best far corrected VA (Snellen)</td>
<td>✓</td>
</tr>
<tr>
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<td>✓</td>
</tr>
<tr>
<td>Slit Lamp Biomicroscopy (anterior health)</td>
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<td>Corneal/conjunctival staining (Lemp scale)</td>
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<td>Non-invasive tear breakup time (NIBUT)</td>
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</tr>
<tr>
<td>Infrared ocular thermography</td>
<td>✓</td>
</tr>
</tbody>
</table>

2.4.3.9 Statistical analysis

Data was tabulated and analysed using various statistical software and presented in Chapter 5, 6 and 7. In total 62 dry eye and 63 age- and sex-matched controls were analysed for Chapter 5 and 6 and 62 dry eye and 82 controls were analysed for Chapter 7.

2.5 References


3 Prevalence of and risk factors for symptomatic dry eye disease in Singapore

Contributions
I designed this study in collaboration with my supervisors and co-authors. I was solely responsible for participant recruitment and data collection. I also analysed the data with useful guidance from my supervisors and Dr. Robert Straughan. I wrote the manuscript with helpful comments from my supervisors and co-authors.

Publications

Conference presentations
18th Asia Pacific Optometric Conference (APOC), 24 – 26th November 2011, Suntec Convention Centre, Singapore (paper presentation).

Acknowledgements
Supported by Singapore ToteBoard Organisation no. LS/CLS/TM/2009/007: two-years research grant 2009 – 2011 under the TOTE Board Model Project at the 40th TOTE Fund Committee Meeting. Six-years tuition grant 2010 – 2016 under the Cat A postgraduate study scheme (University of Manchester PhD study) awarded by Singapore Polytechnic. The authors thank Matthew Ta Yu Ze, Leow Zhun Hong, Chong Jia Sheng and Ting Wei Min in helping to interview participants at various MRT stations.
3.1 Abstract

3.1.1 Purpose
To describe the prevalence and risk factors of symptomatic dry eye disease (SDED) in Singapore.

3.1.2 Methods
A cross-sectional dry eye survey was carried out using the McMonnies dry eye questionnaire. Members of the public were interviewed at the 46 (out of 62) randomly selected mass rapid transit (MRT) stations and their vicinity. A total of 1004 questionnaires were collected for participants aged between 15 and 83 years old. Symptomatic dry eye disease (SDED) was defined as those with at least one of five self-reported symptoms that were reported as often or constantly. Non-dry eye (NDE) subjects were those with no related symptoms reported. Prevalence of SDED in the studied population and confidence interval (CI) were calculated. Risk factors were also evaluated using logistic regression analysis at 95% CI.

3.1.3 Results
The prevalence for SDED was found to be 12.3% with prevalence greater in females than males. SDED was shown to be significantly associated with contact lens wear (odds ratio [OR] 2.96, 95% CI: 1.81 – 4.83), those having had previous treatment for dry eye (OR 2.09, 95% CI: 1.33 – 3.29), those taking medication (OR 1.84, 95% CI: 0.99 – 3.44), those with unusual sensitivity of eyes (OR 3.04, 95% CI: 1.92 – 4.83), constant mucous membrane dryness (OR 4.11, 95% CI: 1.62 – 10.45), and irritation on waking (OR 2.38, 95% CI: 1.34 – 4.22). Smoking was not found to be associated with SDED.

3.1.4 Conclusions
Singapore has SDED prevalence of 12.3% and was associated with contact lens wear, those had previous treatment in dry eye, medication, those having unusual sensitivity of eyes, mucous membrane dryness and waking irritation. These new data will be of value to the eyecare community in Singapore and elsewhere.

Keywords: Prevalence, symptomatic dry eye, McMonnies Dry Eye Questionnaire, risk factors
3.2 Introduction

Dry eye disease (DED) is well recognised as a global public health problem affecting millions of people because of its high prevalence and morbidity (Lemp, 1995, Bandeen-Roche et al., 1997, Brewitt and Sistani, 2001, Begley et al., 2001, Schaumberg et al., 2002). DED prevalence as documented in large epidemiological studies ranges from 5% (McCarty et al., 1998) to over 30% (Lin et al., 2003). DED has significant socio-economic implications such as increased health care costs and a negative impact on vision-related quality-of-life issues such as driving, television watching, reading, computer work and emotional well-being (Miljanovic et al., 2007, Tong et al., 2010, Pouyeh et al., 2012). Evaluating the cost of DED treatment is problematic in view of the multifactorial nature of the condition. The cost of managing DED in health care organisations in the United States has been estimated at US $700,000 per million patients (Munoz et al., 2000). Furthermore, the total annual healthcare cost has been reported to range from US$0.27 million in France to US$1.10 million in the UK for every 1,000 DED patients managed by ophthalmologists (Clegg et al., 2006). A two year retrospective study at the Singapore National Eye Centre (SNEC) on 54,052 patients reported an total annualised cost of dry eye treatment about US$1.5 million for 2008 and 2009 (Waduthantri et al., 2012).

There is lack of published data on dry eye prevalence in Singapore. Tong et al. (2009) reported a prevalence of 6.5% with association to cigarette smoking, presence of thyroid disease and higher income. However, this work only investigated patients of Malay ethnicity aged 40-80 selected from south-western Singapore (Tong et al., 2009). As Singapore is a multiracial country, consisting of three main ethnic groups (Chinese, Malays and Indians), this previous study was repeated and expanded. To that end, the current study was designed to estimate the prevalence and report the risk factors for DED across all ethnic groups in Singapore, inclusive of a wide age range and both genders.

3.3 Methods

3.3.1 Population study

Sample size calculation

This cross-sectional study was carried out May to August 2010. Before commencement, the appropriate sample size was determined as follows. Assuming a margin of error of ± 5% and (p) as the estimated prevalence of dry eye in Singapore, the sample size needed for this study was:

\[ n = \frac{p \times (1-p) \times (1.96/0.05)^2}{(at \ 95\% \ confidence \ level)} \]

Assuming a worst case scenario of \( p = 0.5 \), the minimum sample size needed for the required margin of error was 385.
Sampling method to represent Singapore population

The Mass Rapid Transport (MRT) is a common mode of transport amongst Singaporeans and is evenly distributed across the island of Singapore and use of MRT stations was chosen as a suitable way to seek out study participants. The passenger numbers for the MRT have been rising steadily and were up to about 39 million per month in 2007 (Chio, 2008).

Forty-six of the 62 MRT stations were randomly selected to be study sites and covered all parts of Singapore (Table 3.1). Members of the public were approached at the MRT stations and in nearby locations such as shopping malls, food centres and shops in order to reach an appropriately representative sample of the Singapore population.

Table 3.1. List of MRT stations selected after randomization.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aljunied</td>
<td>16.</td>
</tr>
<tr>
<td>12.</td>
<td>Chinatown</td>
<td>27.</td>
</tr>
<tr>
<td>13.</td>
<td>Chinese Garden</td>
<td>28.</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

In common with other epidemiological studies of symptomatic diseases, a symptom questionnaire was employed in this study, with the McMonnies dry eye questionnaire (DEQ) (appendix 3.1) selected as the best available questionnaire. This questionnaire is a long-standing and widely used DEQ for screening dry eye disease (DED) and reported sensitivity of 87% to 98% and specificity 87% to 97% (Golding and Brennan, 1993, McMonnies and Ho, 1987, McMonnies et al., 1998). Although Rasch analysis using the DEQ on 43 female Sjögren syndrome patients (> 45 years) and
140 age-matched controls showed that the questionnaire cannot be used to grade dry eye severity, various scoring methods can be employed with this questionnaire for it to efficiently screen for DED (Gothwal et al., 2010). Indeed, it is regarded as the "gold standard" questionnaire for dry eye and is statistically reliable and repeatable (Erickson et al., 2002).

Members of the public were approached and invited to complete the McMonnies DEQ. The participants were interviewed in either English or Mandarin. Surveyors were conversant in both languages, and the participant’s responses were translated back into English to ensure that there were no discrepancies. The majority of the participants could readily comprehend English.

### 3.3.2 Symptoms and risk factors assessment

In this study, patients with symptomatic dry eye disease (SDED) was defined as those with at least one of five self-reported symptoms (in question two and three of the McMonnies DEQ - soreness, scratchiness, dryness, grittiness and burning sensation) that were reported as ‘often’ or ‘constantly’. In previous studies using the McMonnies DEQ for estimates of dry eye prevalence (Albietz, 2000, Chia et al., 2003, Rege et al., 2013), similar criteria have been employed (Lee et al., 2002, Tong et al., 2009, 2010). Participants who reported the frequency of these symptoms as never or sometimes were considered as non-dry eye (NDE) subjects. The risk factors evaluated were age, gender, ethnicity (race), contact lens wear, medication, smoking, alcohol use, arthritis, thyroid abnormality, nocturnal lagophthalmos and any reported previous treatment of dry eye, unusual sensitivity of eyes, swimming irritation of eyes, mucous membrane dryness and waking irritation as stated in the McMonnies DEQ. All participants were invited to complete the McMonnies DEQ (appendix 3.1) that consists of the relevant questions/risk factors (McMonnies and Ho, 1987) and scoring was done using DEWS dry eye diagnostic template (DEWS, 2007b) (appendix 3.2).

The prevalence of SDED in the studied population and confidence interval (CI) were calculated for the 15 studied risk factors. Potential risk factors were then ruled out (those with p ≤ 0.06) and further evaluated using logistic regression analysis to rule out the ‘true’ risk factors at 95% CI.

### 3.4 Results

A total of 1,004 questionnaires were collected and consisted of different races. The age of the participants ranged between 15 to 83 years old, and was categorized into 3 different age groups (< 25, 25 to 45, and > 45) following recommendation made by McMonnies and Ho (1986).

Table 3.2 shows the demographic of the 1004 participants. 443 (44.1%) of the participants were males and 561 (55.9%) were females. The mean age for the population was 38.2 ± 15.5 years (males: 37.7 ± 15.9 years and females: 38.6 ± 15.1 years). All participants were residents of Singapore. The demographic features closely matched those of the general Singapore population (Singstat, 2009) (Table 3.3).
Table 3.2. Demographic of the 1004 participants.

<table>
<thead>
<tr>
<th></th>
<th>Total Population (n = 1004)</th>
<th>No. of Males (n = 443)</th>
<th>No. of Females (n = 561)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>443 (44.1%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>561 (55.9%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>252 (25.1%)</td>
<td>121 (27.3%)</td>
<td>131 (23.3%)</td>
</tr>
<tr>
<td>25-45</td>
<td>404 (40.2%)</td>
<td>168 (37.9%)</td>
<td>236 (42.1%)</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>348 (34.7%)</td>
<td>154 (34.8%)</td>
<td>194 (34.6%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>756 (75.3%)</td>
<td>336 (75.8%)</td>
<td>420 (74.9%)</td>
</tr>
<tr>
<td>Malay</td>
<td>118 (11.8%)</td>
<td>48 (10.8%)</td>
<td>70 (12.5%)</td>
</tr>
<tr>
<td>Indian</td>
<td>94 (9.4%)</td>
<td>45 (10.2%)</td>
<td>49 (8.7%)</td>
</tr>
<tr>
<td>Others</td>
<td>36 (3.6%)</td>
<td>14 (3.2%)</td>
<td>22 (3.9%)</td>
</tr>
</tbody>
</table>

Table 3.3. Demographic of Singapore population (Singstat, 2009).

<table>
<thead>
<tr>
<th></th>
<th>Total Population (n = 3,733.9)</th>
<th>No. of Males (n = 1,844.7)</th>
<th>No. of Females (n = 1,889.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,844.7 (49.4%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>1,889.1 (50.6%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>1,172.4 (31.4%)</td>
<td>596.3 (32.3%)</td>
<td>576.1 (30.5%)</td>
</tr>
<tr>
<td>25-45</td>
<td>1,201.8 (32.2%)</td>
<td>585.6 (31.7%)</td>
<td>616.3 (32.6%)</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>1,359.6 (36.4%)</td>
<td>662.8 (35.9%)</td>
<td>696.8 (36.9%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>2,770.3 (74.2%)</td>
<td>1,360.2 (73.7%)</td>
<td>1,410.1 (74.6%)</td>
</tr>
<tr>
<td>Malay</td>
<td>500.1 (13.4%)</td>
<td>249.1 (13.5%)</td>
<td>250.9 (13.3%)</td>
</tr>
<tr>
<td>Indian</td>
<td>343.5 (9.2%)</td>
<td>178.1 (9.7%)</td>
<td>165.4 (8.7%)</td>
</tr>
<tr>
<td>Others</td>
<td>120.0 (3.2%)</td>
<td>57.3 (3.1%)</td>
<td>62.7 (3.3%)</td>
</tr>
</tbody>
</table>

3.4.1 Population study

The prevalence of SDED was found to be 12.3% (95% CI, 10.3 - 14.4), which can be extrapolated to approximately 0.5 million out of a total population of close to 4 million Singaporeans. 8.3% of participants described the frequency of their symptoms as ‘constantly’ with the remaining 4.0% as ‘often’. 87.7% of the population studied was found to be NDE; 40.7% of them never experienced any of the dry eye symptoms whereas 48.0% of them had the symptoms occasionally (sometimes) (Fig. 3.1).
Table 3.4 describes the prevalence of SDED in the studied population. Prevalence of females with SDED was 14.8% (95% CI, 12.0 - 18.0) as compared to 9.0% (95% CI, 6.5 - 12.1) in males and the difference was statistically significant (p = 0.006). Other factors that were found to be significantly associated with SDED were: contact lens wear (p < 0.0005), previous dry eye treatment (p < 0.0005), unusual sensitivity of eyes (p < 0.0005), alcohol use (p = 0.062), medication side effects (p = 0.014), mucous membrane dryness (p < 0.0005) and waking irritation (p < 0.0005).

There was a superficial positive correlation between age and the increasing prevalence of SDED. The old age group (> 45 years old) was found to have the highest prevalence rate, 13.8% (95% CI, 10.4 - 17.9), followed by 12.6% (95% CI, 9.6 - 16.3) in the mid age group (25 - 45 years old) and 9.5% (95% CI, 6.2 - 13.8) in the young age group (< 25 years old). However, these differences were not statistically significant (p = 0.277).

Figure 3.1. Prevalence of SDED in the study population.
<table>
<thead>
<tr>
<th></th>
<th>Total Number</th>
<th>N (%)</th>
<th>95 % CI</th>
<th>p-value (Pearson’s chi-square)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All persons</td>
<td>1004</td>
<td>123 (12.3)</td>
<td>10.3 - 14.4</td>
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</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>561</td>
<td>83 (14.8)</td>
<td>12.0 - 18.0</td>
<td>0.006</td>
</tr>
<tr>
<td>Male</td>
<td>443</td>
<td>40 (9.0)</td>
<td>6.5 - 12.1</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young (&lt; 25)</td>
<td>252</td>
<td>24 (9.5)</td>
<td>6.2 - 13.8</td>
<td>0.277</td>
</tr>
<tr>
<td>Mid (25-45)</td>
<td>404</td>
<td>51 (12.6)</td>
<td>9.6 - 16.3</td>
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</tr>
<tr>
<td>Old (&gt; 45)</td>
<td>348</td>
<td>48 (13.8)</td>
<td>10.4 - 17.9</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>756</td>
<td>95 (12.6)</td>
<td>10.3 - 15.1</td>
<td>0.677</td>
</tr>
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<td>Malay</td>
<td>118</td>
<td>16 (13.6)</td>
<td>8.0 - 21.1</td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td>94</td>
<td>8 (8.5)</td>
<td>3.8 - 16.1</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>36</td>
<td>4 (11.1)</td>
<td>3.1 - 26.1</td>
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</tr>
<tr>
<td>Smoker</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>133</td>
<td>15 (11.3)</td>
<td>6.5 - 17.9</td>
<td>0.713</td>
</tr>
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<td>871</td>
<td>108 (12.4)</td>
<td>(0.3 - 14.8)</td>
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<td>Contact Lens Wear</td>
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<tr>
<td>Yes</td>
<td>203</td>
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<td>801</td>
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<td>(8.2 - 12.6)</td>
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<td>Previous Tx of DE</td>
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</tr>
<tr>
<td>Yes</td>
<td>249</td>
<td>56 (22.5)</td>
<td>(17.5 - 28.2)</td>
<td>&lt; 0.0005</td>
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<tr>
<td>No</td>
<td>729</td>
<td>62 (8.5)</td>
<td>(6.6 - 10.8)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>26</td>
<td>5 (1.9)</td>
<td>(0.7 - 3.9)</td>
<td></td>
</tr>
<tr>
<td>Unusual sensitivity of eyes</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>295</td>
<td>65 (22.0)</td>
<td>(17.4 - 27.2)</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>No</td>
<td>537</td>
<td>40 (7.5)</td>
<td>(5.4 - 10.0)</td>
<td></td>
</tr>
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<td>Sometimes</td>
<td>172</td>
<td>18 (10.5)</td>
<td>(6.3 - 16.0)</td>
<td></td>
</tr>
<tr>
<td>Swimming irritation of eyes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>155</td>
<td>21 (13.6)</td>
<td>(8.6 - 20.0)</td>
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</tr>
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<td>(10.2 - 15.5)</td>
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<tr>
<td>Sometimes</td>
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<td>15 (9.5)</td>
<td>(5.4 - 15.2)</td>
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<td>Alcohol use</td>
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<td></td>
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</tr>
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<td>Sometimes</td>
<td>32</td>
<td>1 (3.1)</td>
<td>(0.1 - 16.2)</td>
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<tr>
<td>Medication side effects</td>
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<td></td>
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<td>No</td>
<td>858</td>
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<td>(3.6 - 19.9)</td>
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<td>Mucous membrane dryness</td>
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</tr>
<tr>
<td>Constantly</td>
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<td>(22.4 - 61.2)</td>
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<tr>
<td>Often</td>
<td>33</td>
<td>14 (42.4)</td>
<td>(25.5 - 60.8)</td>
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<tr>
<td>Sometimes</td>
<td>343</td>
<td>31 (9.0)</td>
<td>(6.2 - 12.6)</td>
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</tr>
<tr>
<td>Never</td>
<td>601</td>
<td>67 (11.2)</td>
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<td>Thyroid abnormality</td>
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<td>34</td>
<td>2 (5.9)</td>
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<td>19</td>
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<td>Nocturnal Lagophthalmos</td>
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<td>Uncertain</td>
<td>129</td>
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<td>(6.1 - 17.5)</td>
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<td>Waking Irritation</td>
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<td>(8.1 - 12.3)</td>
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<tr>
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<td>73</td>
<td>14 (19.2)</td>
<td>(10.9 - 30.1)</td>
<td></td>
</tr>
</tbody>
</table>

CI = confidence interval

For ethnicity, the Malays and Chinese reported the numerically highest prevalence of 13.6% (95% CI, 8.0 - 21.1) and 12.6% (95% CI, 10.3 - 15.1) respectively, followed by others (Americans, Australians, Indonesians, Filipinos etc) with the prevalence of 11.1% (95% CI, 3.1 - 26.1) with the lowest prevalence for Indians at 8.5% (95% CI, 3.8 - 16.1) (Figure 3.2). Again, these differences
were not statistically significant \( (p = 0.677) \). Smoking, swimming irritation of eyes, arthritis, thyroid abnormality and nocturnal lagophthalmos were not shown to be associated with SDED.

Figure 3.2. Influence of ethnicity (race) \( (n = 756 \) for Chinese; \( 118 \) for Malay; \( 94 \) for Indian and \( 36 \) for others).

3.4.2 Symptoms and risk factors assessment

The profiling of symptoms reported is shown in Table 3.5. Dryness, scratchiness and soreness were the three most commonly reported symptoms in the studied population. 412 individuals reported dryness with 310 \( (75.2\%) \) of them experienced it sometimes, 60 \( (14.6\%) \) experienced it often and 40 \( (9.7\%) \) experience it constantly. 284 individuals reported scratchiness with 224 \( (78.9\%) \), 37 \( (13\%) \) and 22 \( (7.8\%) \) participants experiencing this symptom sometimes, often and constantly, respectively. 187 individuals reported soreness with 150 \( (80.2\%) \) sometimes, 26 \( (13.9\%) \) often and 11 \( (5.9\%) \) constantly. Only 81 individuals reported grittiness and 89 individuals reported a burning sensation. No participants reported all five symptoms.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Total Number</th>
<th>Never</th>
<th>Sometimes</th>
<th>Often</th>
<th>Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dryness</td>
<td>412</td>
<td>2 (0.5)</td>
<td>310 (75.2)</td>
<td>60 (14.6)</td>
<td>40 (9.7)</td>
</tr>
<tr>
<td>Scratchy</td>
<td>284</td>
<td>1 (0.4)</td>
<td>224 (78.9)</td>
<td>37 (13.0)</td>
<td>22 (7.8)</td>
</tr>
<tr>
<td>Sore</td>
<td>187</td>
<td>0 (0)</td>
<td>150 (80.2)</td>
<td>26 (13.9)</td>
<td>11 (5.9)</td>
</tr>
<tr>
<td>Grittiness</td>
<td>81</td>
<td>0 (0)</td>
<td>49 (60.5)</td>
<td>17 (21.0)</td>
<td>15 (18.5)</td>
</tr>
<tr>
<td>Burning</td>
<td>89</td>
<td>1 (1.1)</td>
<td>62 (69.7)</td>
<td>16 (18.0)</td>
<td>10 (11.2)</td>
</tr>
</tbody>
</table>
Table 3.6. Logistic Regression Analysis for all potential risk factors for SDED.

<table>
<thead>
<tr>
<th>Factors</th>
<th>p-value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.38</td>
<td>0.82</td>
<td>0.52 - 1.28</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young (&lt; 25)</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid (25 - 45)</td>
<td>0.41</td>
<td>1.27</td>
<td>0.72 - 2.24</td>
</tr>
<tr>
<td>Old (&gt; 45)</td>
<td>0.36</td>
<td>1.35</td>
<td>0.71 - 2.56</td>
</tr>
<tr>
<td><strong>Contact Lens Wear</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>&lt; 0.0005</td>
<td>2.96</td>
<td>1.81 - 4.83</td>
</tr>
<tr>
<td><strong>Previous Tx of DE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.001</td>
<td>2.09</td>
<td>1.33 - 3.29</td>
</tr>
<tr>
<td>Uncertain</td>
<td>0.15</td>
<td>2.27</td>
<td>0.75 - 6.89</td>
</tr>
<tr>
<td><strong>Unusual sensitivity of eyes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>&lt; 0.0005</td>
<td>3.04</td>
<td>1.92 - 4.83</td>
</tr>
<tr>
<td>Sometimes</td>
<td>0.13</td>
<td>1.64</td>
<td>0.87 - 3.10</td>
</tr>
<tr>
<td><strong>Alcohol use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.43</td>
<td>1.49</td>
<td>0.55 - 4.04</td>
</tr>
<tr>
<td>Sometimes</td>
<td>0.26</td>
<td>0.31</td>
<td>0.04 - 2.37</td>
</tr>
<tr>
<td><strong>Medication side effects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.05</td>
<td>1.84</td>
<td>0.99 - 3.44</td>
</tr>
<tr>
<td><strong>Mucous membrane dryness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>0.003</td>
<td>4.11</td>
<td>1.62 - 10.45</td>
</tr>
<tr>
<td>Often</td>
<td>0.001</td>
<td>3.97</td>
<td>1.73 - 9.12</td>
</tr>
<tr>
<td>Sometimes</td>
<td>0.06</td>
<td>0.62</td>
<td>0.38 - 1.02</td>
</tr>
<tr>
<td><strong>Waking Irritation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.003</td>
<td>2.38</td>
<td>1.34 - 4.22</td>
</tr>
<tr>
<td>Uncertain</td>
<td>0.18</td>
<td>1.63</td>
<td>0.80 - 3.31</td>
</tr>
</tbody>
</table>

CI = confidence interval

Of all the 15 studied risk factors, eight were found to be ‘potential’ risk factors for SDED in the initial univariate analysis: gender, contact lens wear, previous treatment for dry eye, unusual sensitivity of the eyes, alcohol use, medication, mucous membrane dryness and irritation on waking.

Table 3.6 describes the final multiple logistic regression of the risk factors. This model confirmed that SDED significantly associated with contact lens wear (odds ratio [OR] 2.96, 95%CI: 1.81 – 4.83), previous treatment for dry eye (OR 2.09, 95%CI: 1.33 – 3.29), medication (OR 1.84, 95%CI: 0.99 – 3.44), unusual sensitivity of the eyes (OR 3.04, 95%CI: 1.92 – 4.83), constant mucous membrane dryness (OR 4.11, 95%CI: 1.62 – 10.45), and irritation on waking (OR 2.38, 95%CI: 1.34 – 4.22). For medication, high blood pressure (HBP) medication in particular was found to be highly associated with SDED (16 out of 71 HBP participants reported SDED) and the difference
was highly significant (p < 0.0001) as compared to those not taking medication. Figure 3.3 summarizes the significant variables associated with risk of SDED.

![Figure 3.3. Risk factors assessment for SDED.](image)

### 3.5 Discussion
This is the first population-based study on symptomatic dry eye (SDED) covering all ethnic groups in Singapore across a wide age range (15 – 83 years old). The prevalence for SDED was found to be 12.3% and was equivalent to about 0.5 million of the Singapore population with females more prevalent than males. The risk factors associated with SDED were contact lens wear, those had previous treatment in dry eye, medication, those having unusual sensitivity of eyes, constant mucous membrane dryness and waking irritation. The results appear to validly represent the Singapore population as the sampling was randomised, well distributed among the MRT stations across the island and the demographic of the 1004 participants were well tallied with the demographic of the Singapore population. With only a small minority Singaporeans driving to work (and thereby not using the MRT systems), this work can be considered to accurately describe the general situation for SDED in the country.
There have been various population-based dry eye-related studies undertaken in other countries using different diagnostic criteria, and the reported prevalence rates for dry eye/tear film disorders varied widely. The Women's Health study (WHS) and the Physician Health's study (PHS) are amongst the largest studies on dry eye prevalence to date. It was reported that 3.2 million women (7.8%) and 1.6 million men (4.7%) in the United States aged 50 years and older had dry eye (Schaumberg et al., 2003, 2009). The prevalence rates reported in the current study were broadly similar to those found in the United States.

An overall summary of data reported in the literature and findings of the current study is shown in Table 3.7a and 3.7b. It must be noted that direct comparisons between this findings and those in the literature are problematic due to differences in: definitions of dry eye/tear film disorders, questionnaires, climates, life styles, ethnicities and age ranges. However, the findings are within the range (5% to 30%) found for other studies (Smith et al., 2007). Association between SDED with age, gender, contact lens wear and medication was also demonstrated in the current study.

Consistent with previous reports, older age (McCarty et al., 1998, Moss et al., 2000, Schaumberg et al., 2003, Chia et al., 2003, Schaumberg et al., 2009) and female gender (Schaumberg et al., 2003, Uchino et al., 2011) were shown to be associated with DED. The Shihpai eye study in Taiwan (Lin et al., 20030 on older age group (≥ 65 years) revealed a high prevalence rate of 33.7% as compared to Sumatra (Lee et al., 2002) and China study (Zhang et al., 2012) on younger age group (≥ 21 years) that reported a lower rate of 27.5% and 23.7% respectively. A positive correlation between age and SDED prevalence was observed in this study but was not significant. It was well recognised that dry eye is more in women than men (Schaumberg et al., 2003, Uchino et al., 2011) and may be related to levels of hormones. For example, androgen deficiency could be an explanation to a higher prevalence of dry eye in women that can be worsen due to aging and menopause. Postmenopausal hormone replacement therapy that is associated with a higher risk of dry eye could be a reason why SDED in women is more prevalent than it is for men (Schaumberg et al., 2000). A more recent study has confirmed that DED was more severe in women and having a greater impact on their self-assessed well-being and dissatisfaction with treatment side effects (Schaumberg et al., 2013).

No differences were demonstrated between the ethnicities evaluated. A prevalence rate for SDED of 13.6% for Malays, about twice that reported by Tong et al. (2009) was found. This figure may be more realistic as it covers more districts in Singapore and a wider age range as compared to 15 residential districts and age range of 40 – 80 in Tong et al. (2009). There is very limited data on potential effect of race or ethnicity on dry eye prevalence to date although the WHS study suggest that the prevalence of dry eye may be greater in Hispanic and Asian, as compared to Caucasian, women (Schaumberg et al., 2003).
**Risk factors**

The three commonly reported symptoms found in the studied population were dryness, scratchiness and soreness. Symptoms have been reported as being associated with poor tear stability (Holly and Lemp, 1973, Bron, 2001, Goto et al., 2003) and ocular surface damage in DED but this is not always the case. It should be noted that symptoms reported can be due to other conditions sometimes (eg. blepharitis etc) and that DED can be symptomless (Smith et al., 2007). Symptom assessment has been recommended as one of the three important aspects when assessing dry eye, besides tear stability/dynamic and ocular surface damage assessment (DEWS, 2007a, Kaercher and Bron, 2008).

Risk factors found to be associated with SDED were contact lens wear, those with previous treatment for dry eye, those having unusual sensitivity of eyes, irritation on waking, mucous membrane dryness and medication.

Dryness related to contact lens wear was first reported in the 1980s (McMonnies and Ho, 1986, Brennan and Efron, 1989) and has been the most frequently reported symptom among contact lens wearers (McMonnies and Ho, 1986). Brennan and Efron (1989) reported 75% of contact lens wearers with dryness in one of the earliest surveys of contact lens associated dryness. They also suggested a possible hormonal influence as an explanation for the higher prevalence in females as compared to males. Using a self-administered questionnaire, Young et al. (2011) reported that 44% of 932 contact lens wearers in the United Kingdom experienced dry eye symptoms. Nichols et al. (2005) studied on a mixed population of 893 patients and found that contact lens wearers were most likely to report dry eye (52.3%), followed by spectacle wearers (23.9%) and clinical emmetropes (7.1%). It was therefore not surprising to see that contact lens wear was a significant risk factor in the current study. Moss et al. (2000) recorded a similar trend with 15.3% of contact lens wearers reporting dry eye symptoms, and 12.8% of non-contact lens wear reporting similarly. Unfortunately, the modality and duration of contact lens wear was not investigated in the current study. It should also be noted that advances in contact lens technology between 1986 and 2005 may or may not be a factor in dry eye symptom prevalence.

The odds of SDED among those who have had previous treatment in dry eye was about two times greater than those without.

Participants with unusual sensitivity of the eyes, mucous membrane dryness or irritation on waking were associated with SDED (Odds for having dry eye were 2.4 to four times greater compared to those not reporting these symptoms). In this study, people who were diagnosed and have had previous treatment in dry eye are shown to be still having symptoms. The underlying reasons for individuals having unusual sensitivity of eyes or irritation on waking having dry eye is complex and multifactorial (Smith et al., 2007). Those with dry eye symptoms and constant mucous membrane dryness could be patients with Sjögren syndrome or those taking medication. Sjögren syndrome
Dry eye is an exocrinopathy in which the lacrimal and salivary glands are targeted by an autoimmune process. Other organs are also likely to be affected. It is due to infiltration of activated T-cells, which cause acinar and ductular cell death and finally hyposecretion of the tears (Albietz et al., 2003). Inflammatory reaction within the glands leads to the introduction of autoantigens at the surface of epithelial cells (Albietz et al., 2003) and the retention of tissue-specific CD4 and CD8 T-cells (Murube and Rivas, 2003). There are two forms of Sjögren Syndrome, primary and secondary. Primary Sjögren Syndrome is the occurrence of ADDE together with symptoms of dry mouth, in the presence of autoantibodies, evidence of reduced salivary secretion (Brignole et al., 2000). Secondary Sjögren Syndrome is the same as primary Sjögren Syndrome but together with the features of an overt autoimmune connective disease, such as rheumatoid arthritis. Dry eye and dry mouth symptoms could also happen in those taking medications particularly the elderly (Schein et al., 1996, 1999). Not many of them would be represented in this study but those items like having arthritis (or any other connective tissue disease) and/or, dry mucous membranes can be useful indicators of the possibility of a Sjögren’s diagnosis when there are symptoms of dry eye.

Singaporeans taking medication particularly those being treated for hypertension were found to be more prone to have symptoms of dry eye and this finding is consistent with recent estimates (Schaumberg et al., 2009). A cross-sectional study on elderly in United States (Schein et al., 1996, 1999) found a significantly higher prevalence of dry eye or dry mouth symptoms in those taking diuretics or other hypertensive medications. Another study found a borderline increase risk of dry eye associated with the use of diuretics but the risk was decreased with use of angiotensin-converting enzyme inhibitors (Moss et al., 2004, 2008). Use of antidepressants was also shown to be strongly associated with dry eye (Schein et al., 1999, Moss et al., 2008, Schaumberg et al., 2009). A cause-effect relationship between medical conditions and the ocular features was not able to confirm since this was a cross-sectional study.

Smoking was found not to be associated with SDED unlike the finding reported by Tong et al. (2009). Reports on the effect of smoking on dry eye had been controversial. Smoking was reported as risk factor for dry eye in one study (Moss et al., 2000) though not in others (Schein et al., 1997, McCarty et al., 1998).

As McMonnies DEQ was used as a tool to reveal risk factors, other possible factors could be ommitted. For example, Meibomian gland disease/dysfunction (MGD) was not specifically evaluated in this study. MGD is one of the most common cause of SDED and is more prevalent in older patients (DEWS, 2007b). Meibomian glands are important in producing tear film lipid layer and maintain its stability. Any problems on the glands would cause tear film instability and promotes evaporative dry eye (Schaumberg et al., 2009) and therefore may account for some of the symptoms found. In other words, although the McMonnies DEQ did not directly ask about MGD, it is represented in the findings. Factors like omega-3 and omega-6 fatty acids (Miljanovic et al., 2005), connective tissue disease, LASIK and refractive excimer laser surgery (Hovanesian and Shah, 2011), radiation therapy, hematopoietic stem
cell transplantation, vitamin A deficiency (Sommer, 2003), Hepatitis C infection (Zegans et al., 2002), androgen deficiency and diabetes mellitus (Kaiserman et al., 2005) that have been reported as possible risk factors for dry eye were not assessed.

3.6 Conclusions

Prevalence of SDED in Singapore population aged 15 to 83 years was 12.3%. Risk factors associated with SDED were contact lens wear, those had previous treatment in dry eye, those having unusual sensitivity of eyes, waking irritation, mucous membrane dryness and medication. This new information about the prevalence of SDED and its risk factors in Singapore will serve to inform the eyecare community and assist them in advising and managing this patient population whose quality of life may be negatively impacted by this disease.
Table 3.7a. Dry eye prevalence reported in the literature & current study.

<table>
<thead>
<tr>
<th>Study &amp; Author</th>
<th>N</th>
<th>Age range (years)</th>
<th>Questionnaire used &amp; mode of study</th>
<th>Prevalence</th>
<th>Conclusion/Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US Studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salisbury Eye Study (Munoz et al., 2000)</td>
<td>2420</td>
<td>≥ 65</td>
<td>At least 1 of 6 symptoms (dryness, gritty/sandiness, burning, redness, crusting on lashes, eye stuck shut in morning), occurring at least often.</td>
<td>14.6%</td>
<td></td>
</tr>
<tr>
<td>Beaver Dam (Moss et al., 2000)</td>
<td>3722</td>
<td>≥ 48</td>
<td>“For the past 3 months or longer have you had dry eyes?” (if needed, Described as foreign body sensation with itching, burning, sandy feeling, not related to allergy).</td>
<td>14.4%</td>
<td></td>
</tr>
<tr>
<td>Women’s Health Study (Schaumberg et al., 2003)</td>
<td>36995</td>
<td>≥ 49</td>
<td>Severe symptoms of dryness and irritation, either constantly or often, and/or the physician’s diagnosis of dry eye as volunteered by the patient.</td>
<td>7.8% (-3.2 million)</td>
<td>Age Ethnicity (more in Hispanic and Asian as compared to Caucasians) Educational level Geographic differences</td>
</tr>
<tr>
<td>Physician’s Health Studies I and II (Miljanovic et al., 2007, Schaumberg et al., 2009)</td>
<td>25655</td>
<td>≥ 50, 55</td>
<td>Severe symptoms of dryness and irritation, either constantly or often, and/or the physician’s diagnosis of dry eye as volunteered by the patient.</td>
<td>4.7% (-1.6 million)</td>
<td>Age Hypertension Benign prostatic hyperplasia Use of antidepressants</td>
</tr>
<tr>
<td><strong>Australian Studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melbourne Visual Impairment Project (McCarty et al., 1998)</td>
<td>926</td>
<td>≥ 40</td>
<td>At least 1 of 6 “severe” symptoms, not attributed by the subject to hay fever (discomfort, foreign body, itching, tearing, dryness, photophobia).</td>
<td>5.5%</td>
<td></td>
</tr>
<tr>
<td>Blue Mountains study (Chia et al., 2003)</td>
<td>1075</td>
<td>≥ 50</td>
<td>At least 1 of 4 symptoms regardless of severity, or at least 1 symptom with a moderate to severe ranking (dryness, grittiness, itchiness, discomfort).</td>
<td>16.6% (at least 1 symptom) 15.3% (3 or more symptoms)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3.7b. Dry eye prevalence reported in the literature & current study (cont.).

<table>
<thead>
<tr>
<th>Study &amp; Author</th>
<th>N</th>
<th>Age range (years)</th>
<th>Questionnaire used &amp; mode of study</th>
<th>Prevalence</th>
<th>Conclusion/Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asian Studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sumatra study (Lee et al., 2002)</td>
<td>1058</td>
<td>≥ 21</td>
<td>At least 1 of 6 symptoms, often or all of the time (dryness, gritty/sandiness, burning, redness, crusting on lashes, eye stuck shut in morning).</td>
<td>27.5%</td>
<td>-</td>
</tr>
<tr>
<td>Shihpai Eye Study (Lin et al., 2003)</td>
<td>2038</td>
<td>≥ 65</td>
<td>At least 1 of 6 symptoms, often or all of the time (dryness, gritty/sandiness, burning, sticky, tearing, redness, discharge, eye stuck shut in morning).</td>
<td>33.7%</td>
<td>-</td>
</tr>
<tr>
<td>Japanese study (Uchino et al., 2011)</td>
<td>3294</td>
<td>≥ 40</td>
<td>Severe symptoms of dryness and irritation, either constantly or often, and/or the physician’s diagnosis of dry eye as volunteered by the patient.</td>
<td>21.6%</td>
<td>Contact lenses Low BMI &amp; Hypertension (men) Myocardial infarction or angina and VDT use (women)</td>
</tr>
<tr>
<td>China study (Zhang et al., 2012)</td>
<td>1902</td>
<td>Senior high school</td>
<td>Severe symptoms of dryness and irritation, either constantly or often, and/or the physician’s diagnosis of dry eye as volunteered by the patient.</td>
<td>23.7%</td>
<td>Myopia Contact lens wear Inadequate refractive correction Use of topical eye drops Poor sleep quality</td>
</tr>
<tr>
<td>Current study (2015)</td>
<td>1007</td>
<td>15 - 83</td>
<td>At least 1 of 5 symptoms, often or constantly (dryness, scratchiness, soreness, grittiness, burning).</td>
<td>12.3% (~ 0.5 million)</td>
<td>Contact lens wear Previous treatment in dry eye Medication Unusual sensitivity of eyes Mucous membrane dryness Waking irritation</td>
</tr>
</tbody>
</table>
3.7 References


4 Repeatability of infrared ocular thermography in assessing healthy and dry eyes

Contributions
I designed this study in collaboration with my supervisors and co-authors. I was solely responsible for participant recruitment and data collection. I also analysed the data with useful guidance from my supervisors. I wrote the manuscript with helpful comments from my supervisors and co-authors.

Publications

Conference presentations
None

Acknowledgements
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4.1 Abstract

4.1.1 Purpose
To investigate the Inter-image, inter-occasion and inter-examiner repeatability of NEC infrared thermo-tracer TH9260 in assessing healthy and dry eyes.

4.1.2 Methods
Ocular surface temperature (OST) was recorded using NEC infrared thermo-tracer TH9260 on 21 healthy and 15 dry eyes. Data from the right eyes were analyzed. Marking of the ocular surface and OST acquisition was performed using a new ‘diamond’ demarcation method. Twelve OST indices were obtained at three different time points: 0 s, 5 s and 10 s. Inter-image, inter-occasion and inter-examiner repeatability of the infrared ocular thermography was evaluated by calculating coefficients of repeatability (COR).

4.1.3 Results
Ten out of the twelve tested OST indices had good repeatability with small inter-image variability (%COR: 0.2 to 0.9), inter-occasion variability (%COR: 2.1 to 3.7) and inter-examiner variability (%COR: 1.5 to 3.7) for the three studied time points. Two of the OST indices (temperature standard deviation of the region of interest and radial temperature difference) had poor repeatability with much larger inter-image variability (%COR: 8.9 to 140.7), inter-occasion variability (%COR: 47.5 to 153.5) and inter-examiner variability (%COR: 54.7 to 142.0) for the three studied time points.

4.1.4 Conclusions
Most of the metrics adopted in this assessment can be considered to be highly repeatable.

Keywords. Repeatability, dry eye, infrared ocular thermography, ocular surface temperature.
4.2 Introduction

Infrared (IR) ocular thermography determines ocular surface temperature (OST) of the eye and pre-orbital skin by measuring the amount of IR radiation emitted from the surface with an infrared thermal imaging camera. Measurements are then processed into a color-coded display image for interpretation and analysis (Morgan et al., 1993). Non-invasive ocular thermography was first introduced in 1968 and was used to evaluate both normal and pathological conditions (Mapstone, 1968a, Mapstone, 1968b, Mapstone, 1968c, Mapstone, 1968d) and later for dry eye (Morgan et al., 1993, Morgan et al., 1995). It has the advantages over a contact device of being non-invasive, rapid and without the risk of trauma and contamination (Mapstone, 1968d).

The methods used in ocular surface marking and OST acquisition reported in the literature have varied widely (Table 3.1) resulting in different OST indices studied. Although automated methods of OST acquisition have occasionally been adopted (Tan et al., 2009b), a more manual approach has generally been employed (Efron et al., 1989, Morgan et al., 1993, Morgan et al., 1995, Cardona et al., 1996, Mori et al., 1997, Morgan et al., 1999, Murphy et al., 1999, Craig et al., 2000, Purslow et al., 2005, Chiang et al., 2006, Galassi et al., 2007, Sodi et al., 2007, Purslow and Wolffsohn, 2007, Acharya et al., 2008, Chang et al., 2008, Ng et al., 2008, Tan et al., 2009b, Kamao et al., 2011., Su et al., 2011) (Table 4.1).
Table 4.1. Ocular surface marking and OST acquisition reported in the literature.

<table>
<thead>
<tr>
<th>Authors</th>
<th>OST acquisition</th>
<th>Ocular surface marking method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efron et al., 1989</td>
<td>Manual</td>
<td>11 points running across the anterior eye</td>
</tr>
<tr>
<td>Morgan PB 1993, Morgan et al., 1995, Morgan PB 1999</td>
<td>Manual</td>
<td>Five 10 x 10 boxes placed in five anatomical locations along horizontal meridian running across the estimated centre of the cornea</td>
</tr>
<tr>
<td>Galassi et al., 2007</td>
<td>Manual</td>
<td>Five points placed on centre of the cornea, internal and external canthi, half-way from the internal canthus and nasal limbus, half-way from the external canthus and temporal limbus</td>
</tr>
<tr>
<td>Sodi et al., 2007</td>
<td>Manual</td>
<td>Five points equally placed along a horizontal line running through centre of the cornea, connecting medial and lateral canthi</td>
</tr>
<tr>
<td>Murphy et al., 1999</td>
<td>Manual</td>
<td>A squared 10 x 10 pixels box placed at the centre of the cornea</td>
</tr>
<tr>
<td>Mori et al., 1997</td>
<td>Manual</td>
<td>A squared 20 x 20 pixels box placed at the centre of the cornea</td>
</tr>
<tr>
<td>Chiang et al., 2006</td>
<td>Manual</td>
<td>An enriched region of 4.4 mm diameter (22 pixels)</td>
</tr>
<tr>
<td>Ng et al., 2008</td>
<td>Manual</td>
<td>A small circle placed at the centre of the cornea</td>
</tr>
<tr>
<td>Cardona et al., 1996</td>
<td>Manual</td>
<td>A circular region placed at the centre of the cornea</td>
</tr>
<tr>
<td>Craig et al., 2000</td>
<td>Manual</td>
<td>Mean of central cornea pixels</td>
</tr>
<tr>
<td>Purslow et al., 2005, Purslow and Wolffsohn 2007</td>
<td>Manual</td>
<td>23 points placed across the anterior eye</td>
</tr>
<tr>
<td>Tan et al., 2009</td>
<td>Manual</td>
<td>20 points placed across the anterior eye, lined up in the shape of “+”</td>
</tr>
<tr>
<td>Chang et al., 2008</td>
<td>Manual</td>
<td>Acquire local temperature of lateral orbit, upper eyelid, caruncle, medial conjunctiva, lateral conjunctiva, lower eyelid and cornea</td>
</tr>
<tr>
<td>Acharya et al., 2008</td>
<td>Semi-auto</td>
<td>Image was manually cropped to consist only of eye, the cornea was then detected by algorithm developed</td>
</tr>
<tr>
<td>Tan et al., 2009</td>
<td>Automated</td>
<td>The eye was localized by genetic snake algorithm, and the cornea diameter and location were derived from the resultant snake points</td>
</tr>
<tr>
<td>Kamao et al., 2011</td>
<td>Manual</td>
<td>Central corneal 4 mm in diameter, Conjunctiva temporal and Conjunctiva nasal (both 2 mm in diameter)</td>
</tr>
<tr>
<td>Su et al., 2011</td>
<td>Manual</td>
<td>ROI (region of interest) determined by four curves connected between four manually set apexes (top and bottom of the eye, left and right corner of the eye)</td>
</tr>
</tbody>
</table>

The magnitude of repeatability is an important consideration for the clinical use of technical and other devices. While dynamic ocular thermography has been reported to have high accuracy and sensitivity (Tan et al., 2009a), little has been reported about its repeatability. The current study was designed to evaluate the repeatability of NEC infrared thermo-tracer TH 9260 in assessing healthy and dry eyes in three different aspects: inter-image, inter-occasion and inter-examiner variability. A newly developed ‘diamond’ demarcation method was used to mark the ocular surface and study twelve OST indices. In common with all scientific evaluations, repeated measures of the ‘diamond’ method in marking the ocular surface and OST acquisition will necessarily varied due to factors such as instrument fluctuation or non-uniformity within or between samples (Bland and Altman, 1986). Such variation is termed measurement error and can be reported by giving the coefficient of
repeatability (COR), defined as the maximum difference likely to occur between two successive measurements (Bland and Altman, 1996).

4.3 Methods

4.3.1 Subjects

The research protocol was approved by the Singapore National Health Group (NHG) Domain-Specific Review Board (DSRB) and the Singapore Polytechnic ethics review committee and the work adhered to the tenets of the Declaration of Helsinki. Twenty-one healthy (41 ± 9 years; 11 Females, 10 Males) and 15 dry eye (45 ± 10 years; 11 Females, 4 Males) subjects were recruited. Informed consent was obtained from each subject at study enrolment. The inclusion criteria for the dry eye subjects were: use of tear replacement therapy and had either a fluorescein tear break-up time of 10 seconds or less (Golding and Brennan, 1993), or a Schirmer I test result of less than 10 mm in 5 min (Morgan et al., 1995) along with presence of corneal or conjunctiva staining. A drop of fluorescein sodium HCL was instilled on the subject’s eye and the cornea and tear film were assessed using cobalt blue light, viewed through a yellow barrier filter (Wratten #12) for fluorescein tear break-up time and corneal/conjunctival staining. All dry eye subjects were screened and diagnosed by an ophthalmologist at Khoo Tech Puat Hospital eye clinic. Healthy subjects were those not using tear replacement therapy and without signs or symptoms of dry eye. All subjects were required to be non-contact lens wearers for at least two years prior to enrolment. Subjects with any anterior ocular anomalies (e.g. current ocular infection, allergy or ptosis), those undergone surgery or taking any medication that could affect the tear film or who were currently pregnant or breastfeeding were excluded.

4.3.2 Procedures

Subjects were refrained from using their eye-drops or eye make-up on the day of measurement. Ocular thermography was performed in real time using an Infrared thermo-tracer (NEC TH 9260) with resolution of 640 (H) x 480 (V) pixels, operational sensitivity of 0.06 °C and frequency of 30 frames per second, detecting infrared radiation between 8 and 14 µm. The emissivity of 0.98 was assumed (Mapstone, 1968d). A standard examination protocol as reported in the literatures (Morgan et al., 1995, Craig et al., 2000, Purslow and Wolffsohn, 2007, Kamao et al., 2011) was adopted. All the measurements were performed from 9 am to 2 pm in the same room with controlled room temperature (24.06 ± 0.41 °C) and humidity (49.76 ± 2.61 %), with no air drifts and same brightness (300 lux). Subjects were adapted to the room for 20 minutes prior to ocular thermography as previous work has shown that this period was necessary to achieve ocular temperature stabilisation (Morgan, 1994). As corneal temperature is strongly associated with body temperature and seemed to plateau at 36.5°C to 37.0°C (Kessel et al., 2010), body temperature for all subjects were measured and subjects with body temperature ≥ 37 °C were excluded. OST was recorded under the conditions described by Morgan and associates: the subjects blink normally, closed for 3 s and the first image was recorded just after the eyes had opened (Morgan et al.,
The time upon eye opening was recorded as 0 s. 300 frames of real time thermal images reflecting OST changes at the ocular surface were captured over 10 s sustained eye opening. The measurement was done three times on right eye followed by left eye. At any time if subject blinked or changed fixation before 10 s, the measurement was discounted and repeated.

To minimize possible inconsistency in OST acquisition due to variation in palpebral aperture size in Asian eyes (the majority of which were narrow), a newly developed method was used in marking the ocular surface / region of interest (ROI) on the computer images which was termed the ‘diamond’ method. In this method, the lower half / inferior zone of the ocular surface was studied (Fig. 4.1) using a custom-designed OST Analysis V2 software (developed using MatLab Simulink 7.11.0, R2010b, appendix 4.1). The inferior zone of the ocular surface has been reported to be a predictive area in the detection of dry eye subtypes (Fenner and Tong, 2013) and is generally not obscured by eyelashes during OST measurement. Using this method, the problem of the truncated image by upper lid (Pattmoller et al., 2014) is also eliminated.

![Image of Ocular Surface Temperature Analysis](image)

**Figure 4.1.** Marking on ocular surface and OST acquisition using the ‘diamond’ method with the OST Analysis V2 software.

The best of the three sets of images was selected subjectively (an unobscured ocular surface and stable fixation was considered optimum). The first frame of the images was selected and five anatomical points (labelled as 1 - 5) of the ocular surface were marked by positioning a movable cross-hair cursor to form the ROI which shaped like a diamond (Fig. 4.1) with point 1 and 2 along horizontal meridian and running across the estimated geometric center of the cornea (GCC) [8]. Each point marked represents an area of 3 × 3 pixels so that temperature was an average of nine pixels:-
1 - Temporal limbus (LT)
2 - Nasal limbus (LN)
3 - Extreme temporal conjunctiva (T1)
4 - Extreme nasal conjunctiva (T4)
5 - Most inferior point of the ocular surface

Once the marking of ROI was completed, OST acquisition and processing was performed automatically by double clicking the last point marked (point 5) to activate the OST Analysis V2 program and process all the 300 frames. All frame marking and data processing were undertaken by a single examiner (LL). Twelve OST indices of the ocular surface were generated as shown in Table 4.2. In this study, GCC denotes the temperature of central cornea, obtained midway between LT and LN. Twelve OST indices were selected to document the whole inferior zone of the exposed ocular surface within ROI.

Table 4.2. The twelve OST indices studied.

<table>
<thead>
<tr>
<th>OST indices</th>
<th>Description</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC</td>
<td>Geometric center of the cornea (midway between LT and LN)</td>
<td>Most commonly studied in the literature</td>
</tr>
<tr>
<td>MOST</td>
<td>Mean OST of the ROI</td>
<td>-</td>
</tr>
<tr>
<td>T1</td>
<td>Extreme temporal conjunctiva</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>Extreme nasal conjunctiva</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Mid-temporal conjunctiva (midway between T1 and LT)</td>
<td>Study of the different areas of the limbus and conjunctiva</td>
</tr>
<tr>
<td>LT</td>
<td>Temporal limbus</td>
<td>Each point marked = area of 3 x 3 pixels</td>
</tr>
<tr>
<td>LN</td>
<td>Nasal limbus</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>Mid-nasal conjunctiva (midway between T4 and LN)</td>
<td></td>
</tr>
<tr>
<td>MinT</td>
<td>Minimum temperature of ROI</td>
<td>Study of the minimum and maximum temperature of the ocular surface</td>
</tr>
<tr>
<td>MaxT</td>
<td>Maximum temperature of ROI</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>Temperature Standard Deviation of the ROI</td>
<td>-</td>
</tr>
<tr>
<td>RTD</td>
<td>Radial Temperature Difference, ((LT + LN)/2 - GCC)</td>
<td>The difference in temperature between the limbus and GCC, across the horizontal meridian of the ocular surface</td>
</tr>
</tbody>
</table>

The twelve OST indices (Table 2) were obtained at three different time points: 0 s, 5 s and 10 s as each time point was considered potentially useful in future work. Coefficient of repeatability (COR) (BSI, 1979) was calculated for each OST index and at each time point for: (1) Inter-image / image analysis repeatability: an estimate of the variability of two repeated markings (at one week interval) using the ‘diamond’ method on the same image. (2) Inter-occasion (Intra-examiner) repeatability: an estimate of the variability when taking repeated measurements at two different occasions. The subjects left the room for 20 min and measurement were repeated as described by a single examiner after the usual 20 min room adaptation (LL). (3) Inter-examiner repeatability: an estimate of the variability when taking repeated measurements by two independent examiners (LL and PF)
at one occasion, one after another. COR and %COR was chosen as the estimate of measurement error as this provides an immediately meaningful indicator, against which absolute measures of the OST indices can be judged.

4.3.3 Data Analysis

Data of the absolute temperature was analysed. To prevent difficulties arising when non-independent data were collected from both eyes, only data obtained from right eye were used in the analysis (Ray and O’Day, 1985). Repeatability of the ocular thermography was evaluated in three aspects:-

1) **Inter-image / Image analysis repeatability.** Images obtained were marked repeatedly and inter-image repeatability was calculated using the method recommended by Bland and Altman (1986) and Bland and Altman (1996). In brief, the COR is $\sqrt{2} \times 1.96 \times S_w$ or $2.77 \times S_w$ where $S_w$ denotes the average standard deviation of two sets of repeated measurement. The COR values estimates the maximum difference likely to occur between 95% of pairs of successive OST measurements on the same image. As ocular thermography were done for 10 s upon eye opening, COR values were calculated separately at three time points (0 s, 5 s and 10 s). This was to take time factor into consideration and evaluate if the variability would change over time. Results were presented in COR (%COR). In addition, paired t-tests were done. Lastly, the mean of the analysis / reanalysis OST was plotted against the difference between these two measurements using the approach suggested by Bland and Altman (1986) to explore the relationship between measurement error and measurement magnitude and to derive the 95% limits of agreement.

2) **Inter-occasion (intra-examiner) repeatability.** This studied the repeatability of the two sets of OST measurements at two different occasions by a single examiner. All three CORs (at 0 s, 5 s and 10 s) were assessed using the same calculation method as described in (1).

3) **Inter-examiner repeatability.** This studied the repeatability of the two sets of OST measurements by two independent examiners at one occasion. Again, all three CORs (at 0 s, 5 s and 10 s) were assessed using the same calculation method as described in (1).

4.4 Results

Inter-image repeatability is summarised in Table 4.3 showing the p values (paired t-test), within-image standard deviation ($S_w$), and COR in absolute terms and as a percentage of the mean value measured. There was no significant difference between the distribution of differences when a set of images were marked repeatedly on all the twelve OST indices studied at 95% CI (paired t-test, p > 0.05) at all the three studied time points: 0 s, 5 s and 10 s (Table 4.3). Image analysis COR was found to be small for ten out of the twelve OST indices, namely GCC, MOST, T1, T4, CT, LT, LN, CN, MinT and MaxT with %COR ranging from 0.2 to 0.9 within the three studied time points. However, COR was found to be much larger for SD and RTD with %COR ranging from 8.9 to 140.7
within the three studied time points. Bland Altman plots for inter-image analysis / reanalysis of the twelve OST indices at 0 s, 5 s and 10 s are shown in Figure 4.2a and 4.2b. The approx. upper and lower 95% limits of agreement and mean difference are shown in the plots. Findings suggested that there was no consistent trend for the differences to alter with increasing mean values for all the twelve OST indices.

A summary of inter-occasion repeatability is shown in Table 4.4 showing the p values (paired t-test), within-occasion standard deviation (Sw), and COR in absolute terms and as a percentage of the mean value measured. There was no significant difference between the distributions of differences for the two sets of OST measurements on all the twelve OST indices studied at 95% CI (paired t-test, p > 0.05) at all the three selected time points: 0 s, 5 s and 10 s (Table 4.4). Similarly, inter-occasion COR was found to be small for ten out of the twelve OST indices, namely GCC, MOST, T1, T4, CT, LT, LN, CN, MinT and MaxT with %COR ranging from 2.1 to 3.7 within the three studied time points. However, COR was found to be much larger for SD and RTD with %COR ranging from 47.5 to 153.5 within the three studied time points. Bland Altman plots for inter-occasion analysis / reanalysis of the twelve OST indices at 0 s, 5 s and 10 s are shown in Figure 4.3a and 4.3b. The approx. upper and lower 95% limits of agreement and mean difference are shown in the plots. Findings suggested that there was no consistent trend for the differences to alter with increasing mean values for all the twelve OST indices.

A summary of inter-examiner repeatability is shown in Table 4.5 showing the p values (paired t-test), within-examiner standard deviation (Sw), and COR in absolute terms and as a percentage of the mean value measured. Again, there was no significant difference between the distributions of differences for the two sets of OST measurements on all the twelve OST indices studied at 95% CI (paired t-test, p > 0.05) at the three selected time points: 0 s, 5 s and 10 s (Table 4.5). Inter-examiner COR was also found to be small for ten out of the twelve OST indices, namely GCC, MOST, T1, T4, CT, LT, LN, CN, MinT and MaxT with %COR ranging from 1.5 to 3.7 within the three studied time points. COR was again found to be much larger for SD and RTD with %COR ranging from 54.7 to 142.0 within the three studied time points. Bland Altman plots for inter-examiner analysis / reanalysis of the twelve OST indices at 0 s, 5 s and 10 s are shown in Figure 4.4a and 4.4b. The approx. upper and lower 95% limits of agreement and mean difference are shown in the plots. Findings suggested that there was no consistent trend for the differences to alter with increasing mean values for all the twelve OST indices.
Table 4.3. Summary of inter-image repeatability: the p values (from paired t-test), within-image standard deviation ($S_w$), and coefficient of repeatability (COR) shown in absolute terms and as a percentage.

<table>
<thead>
<tr>
<th>OST indices</th>
<th>Overall mean (range)</th>
<th>$p$ values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
<th>Overall mean (range)</th>
<th>$p$ values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
<th>Overall mean (range)</th>
<th>$p$ values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC</td>
<td>34.23 (32.10 to 35.45)</td>
<td>0.98</td>
<td>0.05</td>
<td>0.2 (0.4)</td>
<td>34.58 (30.60 to 36.61)</td>
<td>1.00</td>
<td>0.04</td>
<td>0.1 (0.3)</td>
<td>33.90 (31.87 to 35.15)</td>
<td>0.98</td>
<td>0.04</td>
<td>0.1 (0.3)</td>
</tr>
<tr>
<td>MOST</td>
<td>34.35 (31.73 to 35.53)</td>
<td>0.95</td>
<td>0.03</td>
<td>0.1 (0.2)</td>
<td>34.61 (30.94 to 36.34)</td>
<td>0.89</td>
<td>0.04</td>
<td>0.1 (0.3)</td>
<td>34.11 (32.10 to 35.39)</td>
<td>0.91</td>
<td>0.05</td>
<td>0.1 (0.4)</td>
</tr>
<tr>
<td>T1</td>
<td>34.67 (30.44 to 36.54)</td>
<td>0.96</td>
<td>0.06</td>
<td>0.2 (0.5)</td>
<td>34.31 (32.06 to 35.97)</td>
<td>0.92</td>
<td>0.06</td>
<td>0.2 (0.5)</td>
<td>34.52 (30.52 to 36.54)</td>
<td>1.00</td>
<td>0.06</td>
<td>0.2 (0.5)</td>
</tr>
<tr>
<td>T4</td>
<td>34.26 (31.87 to 35.55)</td>
<td>0.87</td>
<td>0.11</td>
<td>0.3 (0.9)</td>
<td>35.23 (33.17 to 36.71)</td>
<td>0.96</td>
<td>0.10</td>
<td>0.3 (0.8)</td>
<td>34.26 (31.83 to 35.56)</td>
<td>0.97</td>
<td>0.10</td>
<td>0.3 (0.8)</td>
</tr>
<tr>
<td>CT</td>
<td>34.44 (31.48 to 35.64)</td>
<td>0.99</td>
<td>0.04</td>
<td>0.1 (0.3)</td>
<td>33.29 (30.52 to 35.03)</td>
<td>0.97</td>
<td>0.05</td>
<td>0.1 (0.4)</td>
<td>34.28 (31.64 to 35.60)</td>
<td>0.94</td>
<td>0.05</td>
<td>0.1 (0.4)</td>
</tr>
<tr>
<td>LT</td>
<td>34.24 (32.14 to 35.33)</td>
<td>0.96</td>
<td>0.07</td>
<td>0.2 (0.5)</td>
<td>34.27 (31.98 to 35.59)</td>
<td>0.92</td>
<td>0.08</td>
<td>0.2 (0.6)</td>
<td>33.99 (32.70 to 35.11)</td>
<td>0.88</td>
<td>0.09</td>
<td>0.2 (0.7)</td>
</tr>
<tr>
<td>LN</td>
<td>34.46 (31.44 to 35.93)</td>
<td>0.96</td>
<td>0.10</td>
<td>0.3 (0.8)</td>
<td>34.33 (31.71 to 35.64)</td>
<td>0.93</td>
<td>0.10</td>
<td>0.3 (0.8)</td>
<td>34.19 (32.21 to 35.80)</td>
<td>0.91</td>
<td>0.11</td>
<td>0.3 (0.9)</td>
</tr>
<tr>
<td>CN</td>
<td>34.72 (30.83 to 36.34)</td>
<td>0.93</td>
<td>0.07</td>
<td>0.2 (0.6)</td>
<td>34.08 (32.48 to 35.18)</td>
<td>0.93</td>
<td>0.09</td>
<td>0.3 (0.7)</td>
<td>34.54 (31.17 to 36.24)</td>
<td>0.89</td>
<td>0.10</td>
<td>0.3 (0.8)</td>
</tr>
<tr>
<td>MinT</td>
<td>33.51 (30.37 to 34.96)</td>
<td>0.94</td>
<td>0.05</td>
<td>0.1 (0.4)</td>
<td>34.07 (32.61 to 35.26)</td>
<td>0.98</td>
<td>0.05</td>
<td>0.1 (0.4)</td>
<td>33.11 (30.48 to 34.84)</td>
<td>0.99</td>
<td>0.07</td>
<td>0.2 (0.6)</td>
</tr>
<tr>
<td>MaxT</td>
<td>35.25 (32.60 to 36.61)</td>
<td>0.95</td>
<td>0.04</td>
<td>0.1 (0.3)</td>
<td>34.20 (32.06 to 35.48)</td>
<td>0.91</td>
<td>0.04</td>
<td>0.1 (0.3)</td>
<td>35.19 (33.06 to 36.57)</td>
<td>0.93</td>
<td>0.05</td>
<td>0.1 (0.4)</td>
</tr>
<tr>
<td>SD</td>
<td>0.35 (0.18 to 0.55)</td>
<td>0.90</td>
<td>0.01</td>
<td>0.0 (11.1)</td>
<td>0.41 (0.18 to 0.72)</td>
<td>0.91</td>
<td>0.01</td>
<td>0.0 (9.2)</td>
<td>0.45 (0.21 to 0.85)</td>
<td>0.92</td>
<td>0.01</td>
<td>0.0 (8.9)</td>
</tr>
<tr>
<td>RTD</td>
<td>0.12 (-0.71 to 0.53)</td>
<td>0.94</td>
<td>0.06</td>
<td>0.2 (140.7)</td>
<td>0.13 (-0.73 to 0.71)</td>
<td>0.89</td>
<td>0.06</td>
<td>0.2 (134.1)</td>
<td>0.20 (-0.77 to 1.12)</td>
<td>0.84</td>
<td>0.06</td>
<td>0.2 (90.4)</td>
</tr>
</tbody>
</table>
Figure 4.2a. Bland Altman plots for inter-image analysis/reanalysis of the 6 OST indices at (open circles) 0 s, (solid circles) 5 s and (triangle) 10 s: GCC; MOST; T1; CT; LT; LN. The y-axis shows the difference in each of the OST index (°C) between measurements, and the x-axis shows the mean sum of the two measurements. The mean differences are shown with stippled lines. The upper line represents the approx. upper 95% limit of agreement and the lower line represents the approx. lower 95% limit of agreement.
Figure 4.2b. Bland Altman plots for inter-image analysis/reanalysis of the 6 OST indices at (open circles) 0 s, (solid circles) 5 s and (triangle) 10 s: CN; T4; MinT; MaxT; SD; RTD. The y-axis shows the difference in each of the OST index (°C) between measurements, and the x-axis shows the mean sum of the two measurements. The mean differences are shown with stippled lines. The upper line represents the approx. upper 95% limit of agreement and the lower line represents the approx. lower 95% limit of agreement.
### Table 4.4. Summary of inter-occasion repeatability: the p values (from paired t-test), within-occasion standard deviation ($S_w$), and coefficient of repeatability (COR) shown in absolute terms and as a percentage.

<table>
<thead>
<tr>
<th>OST indices</th>
<th>Overall mean (range)</th>
<th>$p$ values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
<th>Overall mean (range)</th>
<th>$p$ values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
<th>Overall mean (range)</th>
<th>$p$ values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC</td>
<td>34.27 (32.87 to 35.44)</td>
<td>0.20</td>
<td>0.34</td>
<td>1.1 (3.2)</td>
<td>33.91 (32.49 to 35.16)</td>
<td>0.48</td>
<td>0.37</td>
<td>1.0 (3.0)</td>
<td>33.72 (31.99 to 35.08)</td>
<td>0.35</td>
<td>0.39</td>
<td>1.1 (3.2)</td>
</tr>
<tr>
<td>MOST</td>
<td>34.57 (33.35 to 35.62)</td>
<td>0.28</td>
<td>0.26</td>
<td>0.9 (2.7)</td>
<td>34.34 (33.10 to 35.40)</td>
<td>0.73</td>
<td>0.31</td>
<td>0.9 (2.5)</td>
<td>34.23 (32.92 to 35.37)</td>
<td>0.59</td>
<td>0.33</td>
<td>0.9 (2.7)</td>
</tr>
<tr>
<td>T1</td>
<td>35.35 (34.32 to 36.36)</td>
<td>0.26</td>
<td>0.24</td>
<td>0.8 (2.2)</td>
<td>35.31 (34.25 to 36.20)</td>
<td>0.40</td>
<td>0.27</td>
<td>0.7 (2.1)</td>
<td>35.24 (33.67 to 36.20)</td>
<td>0.32</td>
<td>0.31</td>
<td>0.9 (2.5)</td>
</tr>
<tr>
<td>T4</td>
<td>34.63 (33.19 to 35.47)</td>
<td>0.48</td>
<td>0.30</td>
<td>1.0 (2.9)</td>
<td>34.68 (33.37 to 35.63)</td>
<td>0.12</td>
<td>0.34</td>
<td>0.9 (2.7)</td>
<td>34.69 (33.57 to 35.70)</td>
<td>0.13</td>
<td>0.34</td>
<td>1.0 (2.7)</td>
</tr>
<tr>
<td>CT</td>
<td>34.75 (33.59 to 35.55)</td>
<td>0.32</td>
<td>0.29</td>
<td>0.9 (2.5)</td>
<td>34.60 (33.26 to 35.61)</td>
<td>0.53</td>
<td>0.34</td>
<td>0.9 (2.7)</td>
<td>34.53 (33.13 to 35.57)</td>
<td>0.57</td>
<td>0.38</td>
<td>1.0 (3.0)</td>
</tr>
<tr>
<td>LT</td>
<td>34.33 (32.49 to 35.27)</td>
<td>0.34</td>
<td>0.38</td>
<td>1.1 (3.3)</td>
<td>34.05 (32.24 to 35.27)</td>
<td>0.96</td>
<td>0.43</td>
<td>1.2 (3.5)</td>
<td>33.92 (32.07 to 35.19)</td>
<td>0.96</td>
<td>0.45</td>
<td>1.2 (3.7)</td>
</tr>
<tr>
<td>LN</td>
<td>34.82 (33.76 to 35.81)</td>
<td>0.06</td>
<td>0.29</td>
<td>1.0 (2.9)</td>
<td>34.56 (33.26 to 35.67)</td>
<td>0.93</td>
<td>0.33</td>
<td>0.9 (2.6)</td>
<td>34.40 (32.98 to 35.57)</td>
<td>0.94</td>
<td>0.37</td>
<td>1.0 (3.0)</td>
</tr>
<tr>
<td>CN</td>
<td>35.28 (34.40 to 36.14)</td>
<td>0.28</td>
<td>0.24</td>
<td>0.8 (2.4)</td>
<td>35.16 (34.17 to 36.29)</td>
<td>0.53</td>
<td>0.29</td>
<td>0.8 (2.3)</td>
<td>35.08 (33.94 to 36.18)</td>
<td>0.65</td>
<td>0.31</td>
<td>0.9 (2.5)</td>
</tr>
<tr>
<td>MinT</td>
<td>33.89 (32.46 to 35.08)</td>
<td>0.21</td>
<td>0.39</td>
<td>1.2 (3.6)</td>
<td>33.53 (31.88 to 34.86)</td>
<td>0.45</td>
<td>0.43</td>
<td>1.2 (3.5)</td>
<td>33.37 (31.41 to 34.84)</td>
<td>0.41</td>
<td>0.44</td>
<td>1.2 (3.7)</td>
</tr>
<tr>
<td>MaxT</td>
<td>35.55 (34.63 to 36.39)</td>
<td>0.43</td>
<td>0.19</td>
<td>0.7 (2.1)</td>
<td>35.50 (34.35 to 36.49)</td>
<td>0.62</td>
<td>0.27</td>
<td>0.7 (2.1)</td>
<td>35.45 (34.38 to 36.51)</td>
<td>0.74</td>
<td>0.29</td>
<td>0.8 (2.2)</td>
</tr>
<tr>
<td>SD</td>
<td>0.34 (0.21 to 0.53)</td>
<td>0.50</td>
<td>0.07</td>
<td>0.2 (47.5)</td>
<td>0.42 (0.24 to 0.77)</td>
<td>0.11</td>
<td>0.09</td>
<td>0.2 (55.8)</td>
<td>0.46 (0.22 to 0.86)</td>
<td>0.12</td>
<td>0.10</td>
<td>0.3 (60.0)</td>
</tr>
<tr>
<td>RTD</td>
<td>0.30 (-0.15 to 0.93)</td>
<td>0.90</td>
<td>0.18</td>
<td>0.5 (153.5)</td>
<td>0.40 (-0.08 to 1.36)</td>
<td>0.10</td>
<td>0.20</td>
<td>0.6 (140.0)</td>
<td>0.44 (-0.01 to 1.49)</td>
<td>0.06</td>
<td>0.22</td>
<td>0.6 (137.0)</td>
</tr>
</tbody>
</table>
Figure 4.3a. Bland Altman plots for inter-occasion analysis/reanalysis of the 6 OST indices at (open circles) 0 s, (solid circles) 5 s and (triangle) 10 s: GCC; MOST; T1; CT; LT; LN. The y-axis shows the difference in each of the OST index (°C) between measurements, and the x-axis shows the mean sum of the two measurements. The mean differences are shown with stippled lines. The upper line represents the approx. upper 95% limit of agreement and the lower line represents the approx. lower 95% limit of agreement.
Figure 4.3b. Bland Altman plots for inter-occasion analysis/reanalysis of the 6 OST indices at (open circles) 0 s, (solid circles) 5 s and (triangle) 10 s: CN; T4; MinT; MaxT; SD; RTD. The y-axis shows the difference in each of the OST index (°C) between measurements, and the x-axis shows the mean sum of the two measurements. The mean differences are shown with stippled lines. The upper line represents the approx. upper 95% limit of agreement and the lower line represents the approx. lower 95% limit of agreement.
Table 4.5. Summary of inter-examiner repeatability: the p values (from paired t-test), within-examiner standard deviation ($S_w$), and coefficient of repeatability (COR) shown in absolute terms and as a percentage.

<table>
<thead>
<tr>
<th>OST indices</th>
<th>Overall mean (range)</th>
<th>p values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
<th>Overall mean (range)</th>
<th>p values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
<th>Overall mean (range)</th>
<th>p values</th>
<th>$S_w$</th>
<th>COR (%COR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC</td>
<td>35.38 (34.32 to 36.29)</td>
<td>0.12</td>
<td>0.35</td>
<td>1.0 (2.9)</td>
<td>34.05 (32.54 to 35.16)</td>
<td>0.10</td>
<td>0.37</td>
<td>1.0 (3.0)</td>
<td>33.86 (31.99 to 35.03)</td>
<td>0.25</td>
<td>0.39</td>
<td>1.1 (3.2)</td>
</tr>
<tr>
<td>MOST</td>
<td>35.30 (34.37 to 36.17)</td>
<td>0.19</td>
<td>0.28</td>
<td>0.8 (2.3)</td>
<td>34.44 (33.23 to 35.40)</td>
<td>0.12</td>
<td>0.31</td>
<td>0.9 (2.5)</td>
<td>34.32 (32.97 to 35.37)</td>
<td>0.27</td>
<td>0.33</td>
<td>0.9 (2.7)</td>
</tr>
<tr>
<td>T1</td>
<td>34.83 (33.76 to 35.81)</td>
<td>0.07</td>
<td>0.24</td>
<td>0.7 (1.8)</td>
<td>35.35 (34.32 to 36.25)</td>
<td>0.10</td>
<td>0.27</td>
<td>0.7 (2.1)</td>
<td>35.29 (33.67 to 36.14)</td>
<td>0.07</td>
<td>0.31</td>
<td>0.9 (2.5)</td>
</tr>
<tr>
<td>T4</td>
<td>35.57 (34.67 to 36.38)</td>
<td>0.22</td>
<td>0.27</td>
<td>0.8 (2.2)</td>
<td>34.64 (33.34 to 35.70)</td>
<td>0.47</td>
<td>0.34</td>
<td>0.9 (2.7)</td>
<td>34.65 (33.56 to 35.61)</td>
<td>0.43</td>
<td>0.34</td>
<td>1.0 (2.7)</td>
</tr>
<tr>
<td>CT</td>
<td>33.91 (32.46 to 35.08)</td>
<td>0.15</td>
<td>0.26</td>
<td>0.7 (2.1)</td>
<td>34.67 (33.34 to 35.56)</td>
<td>0.09</td>
<td>0.34</td>
<td>0.9 (2.7)</td>
<td>34.58 (33.28 to 35.52)</td>
<td>0.13</td>
<td>0.38</td>
<td>1.0 (3.0)</td>
</tr>
<tr>
<td>LT</td>
<td>34.65 (33.34 to 35.67)</td>
<td>0.32</td>
<td>0.33</td>
<td>0.9 (2.7)</td>
<td>34.16 (32.43 to 35.05)</td>
<td>0.06</td>
<td>0.43</td>
<td>1.2 (3.5)</td>
<td>34.02 (32.07 to 35.02)</td>
<td>0.12</td>
<td>0.45</td>
<td>1.2 (3.7)</td>
</tr>
<tr>
<td>LN</td>
<td>34.79 (33.60 to 35.73)</td>
<td>0.12</td>
<td>0.29</td>
<td>0.8 (2.3)</td>
<td>34.67 (33.61 to 35.55)</td>
<td>0.03</td>
<td>0.33</td>
<td>0.9 (2.6)</td>
<td>34.52 (32.98 to 35.53)</td>
<td>0.04</td>
<td>0.37</td>
<td>1.0 (3.0)</td>
</tr>
<tr>
<td>CN</td>
<td>34.36 (33.15 to 35.32)</td>
<td>0.17</td>
<td>0.22</td>
<td>0.6 (1.7)</td>
<td>35.21 (34.21 to 35.99)</td>
<td>0.08</td>
<td>0.29</td>
<td>0.8 (2.3)</td>
<td>35.12 (33.94 to 35.91)</td>
<td>0.17</td>
<td>0.31</td>
<td>0.9 (2.5)</td>
</tr>
<tr>
<td>MinT</td>
<td>34.28 (32.87 to 35.44)</td>
<td>0.26</td>
<td>0.39</td>
<td>1.1 (3.2)</td>
<td>33.69 (31.88 to 34.91)</td>
<td>0.12</td>
<td>0.43</td>
<td>1.2 (3.5)</td>
<td>33.53 (31.41 to 34.77)</td>
<td>0.26</td>
<td>0.44</td>
<td>1.2 (3.7)</td>
</tr>
<tr>
<td>MaxT</td>
<td>34.59 (33.35 to 35.62)</td>
<td>0.17</td>
<td>0.19</td>
<td>0.5 (1.5)</td>
<td>35.53 (34.70 to 36.31)</td>
<td>0.19</td>
<td>0.27</td>
<td>0.7 (2.1)</td>
<td>35.49 (34.70 to 36.20)</td>
<td>0.21</td>
<td>0.29</td>
<td>0.8 (2.2)</td>
</tr>
<tr>
<td>SD</td>
<td>0.34 (0.18 to 0.59)</td>
<td>0.45</td>
<td>0.07</td>
<td>0.2 (54.7)</td>
<td>0.39 (0.18 to 0.77)</td>
<td>0.51</td>
<td>0.09</td>
<td>0.2 (55.8)</td>
<td>0.43 (0.17 to 0.86)</td>
<td>0.79</td>
<td>0.10</td>
<td>0.3 (60.0)</td>
</tr>
<tr>
<td>RTD</td>
<td>0.32 (-0.08 to 0.83)</td>
<td>0.31</td>
<td>0.16</td>
<td>0.5 (142.0)</td>
<td>0.37 (-0.08 to 0.93)</td>
<td>0.50</td>
<td>0.20</td>
<td>0.6 (140.0)</td>
<td>0.41 (-0.01 to 0.98)</td>
<td>0.23</td>
<td>0.22</td>
<td>0.6 (137.0)</td>
</tr>
</tbody>
</table>
Figure 4.4a. Bland Altman plots for inter-examiner analysis/reanalysis of the 6 OST indices at (open circles) 0 s, (solid circles) 5 s and (triangle) 10 s: GCC; MOST; T1; CT; LT; LN. The y-axis shows the difference in each of the OST index (°C) between measurements, and the x-axis shows the mean sum of the two measurements. The mean differences are shown with stippled lines. The upper line represents the approx. upper 95% limit of agreement and the lower line represents the approx. lower 95% limit of agreement.
Figure 4.4b. Bland Altman plots for inter-examiner analysis/reanalysis of the 6 OST indices at (open circles) 0 s, (solid circles) 5 s and (triangle) 10 s: CN; T4; MinT; MaxT; SD; RTD. The y-axis shows the difference in each of the OST index (°C) between measurements, and the x-axis shows the mean sum of the two measurements. The mean differences are shown with stippled lines. The upper line represents the approx. upper 95% limit of agreement and the lower line represents the approx. lower 95% limit of agreement.
4.5 Discussion

The novel ‘diamond’ method was developed for several reasons: (1) to overcome problems of the truncated image by upper lids reported previously (Pattmoller et al., 2014) and (2) to minimize possible inconsistency in OST acquisition due to variation in palpebral aperture size particularly in Asian eyes and (3) to enable study of the inferior zone of the ocular surface which was reported to be a predictive area in the detection of dry eye subtypes (Fenner and Tong, 2013) and is generally not obscured by eyelashes during ocular thermography. OST study was reported to be challenging as the limbal line could not be clearly shown in an ocular thermal image and has made it difficult to locate the boundary of cornea (Tan et al., 2009b). Even though the thermo-tracer used in the current study had reasonably high resolution, marking of the anatomical structures of the ocular surface was still difficult. Using visible light image to superimpose with the infrared thermal image can be a possible strategy to overcome the limitation and serves as a mean for future research.

This is the first study of the repeatability of NEC thermo-tracer TH 9260 in assessing both healthy and dry eyes. The thermography measurement was fast and non-invasive. No reports of reflex tearing and any discomfort from the subjects during the course of the study. Although eight seconds of sustained eye opening has been reported to be an easily achievable target for subjects without causing reflex tearing (Purslow et al., 2005), other studies had reported to be equally achievable for subjects to hold for 10 s (Mori et al., 1997, Kamao et al., 2011).

Ten out of the twelve tested OST indices had shown to be repeatable in terms of inter-image, inter-occasion and inter-examiner analysis. These were valid as the two sets of measurement have presented with small %COR at 95% CI, small average standard deviation (Sw) values in relative to their mean absolute values and there was no consistent trend for the differences to alter with increasing mean values in Bland-Altman plots at 95% CI. %COR can be written in the expression of %COR/mean measurement and small values are considered acceptable (Bland and Altman, 1996). The temperature distribution has been reported to vary across the ocular surface due to tear film distribution and tear film stability depends significantly on lipid layer distribution and causes variation in OST (Craig et al., 2000). In view of the dynamic nature of tear film that change over time and influence OST (Craig et al., 2000), it is essential to study how repeatability can be affected over time. The results showed that time did not influence much on the repeatability of ocular thermography and when both healthy and dry eye subjects hold blink for 10 s, the measurement was repeatable.

To date, there were two inter-occasion variability studies on healthy eyes using different thermo-tracer. THI 500 (Koçak et al., 1999) and Tomey TG 1000 (Klamann et al., 2012) were used respectively with limited OST indices included. In this study, the temperature at the geometric center of the cornea (GCC) has shown to be repeatable and this was in agreement with Koçak et al. (1999)’s study. The minimum (MinT), maximum (MaxT) and mean temperature (MOST) within the ROI were also repeatable and the findings were in agreement with Klamann et al. (2012)’s
study. Subsequently, study of the different areas of the ocular surface as marked as T1, T4, CT, LT, LN and CN (indicating areas of the limbus and conjunctiva) were also shown to be repeatable in the current study. Both Koçak et al. (1999)'s and Klamann et al. (2012)'s studies did not specify the method in ocular surface marking and therefore comparison cannot be made in this aspect.

Inter-image, inter-occasion and inter-examiner repeatability was poor for temperature standard deviation (SD) of the ROI and radial temperature difference (RTD) ie., temperature difference between the limbus and GCC across the horizontal meridian of ocular surface. The two OST indices had presented with large %COR indicating that the test-retest variability was unacceptable at 95% CI. RTD has been studied in the literatures (Morgan et al., 1993, Morgan et al., 1995) and reported to be greater in dry eye as compared to healthy eyes but there were no reports on the repeatability of the thermo-tracer used (IR thermographer NEC6T62). The %COR was largest for RTD (as compared to other OST indices) and with referring to the Bland-Altman plots, the variability of RTD was larger than SD in all the three aspects of analysis (inter-image, inter-occasion and inter-examiner). RTD was calculated from the formula (LT + LN)/2 – GCC which involved three variables and could be the reason how it created variability. It is a condition of “variable within variables”. SD measured the temperature standard deviation of the ROI which could be highly affected by uneven tear film distribution across the ocular surface (Craig et al., 2000) and caused the variation.

The current study has made few improvements on the methodologies. First of all, inter-image and inter-examiner analysis have been included in the analysis instead of just inter-occasion alone. In addition, both dry eye and healthy subjects were studied rather than just healthy eyes in the literatures (Kocak et al., 1999, Klamman et al., 2012). Secondly, the ‘diamond’ demarcation method in marking the ocular surface had overcome the problems of truncated image by upper lid (Pattmoller et al., 2014) that is more apparent for Asian eyes with relatively narrow aperture. Last but not least, the whole of the exposed inferior zone of the ocular surface within ROI and how it changed over time had been studied by including twelve OST indices and investigated over three time points. The findings of this study will determine the direction of future investigations on healthy and dry eyes (Tan et al., 2016).

4.6 Conclusions

The current work described the repeatability of ocular thermography using NEC thermo-tracer TH 9260. Ten out of the twelve OST indices (GCC, MOST, T1, T4, CT, LT, LN, CN, MinT and MaxT) were found to be repeatable when measured at three different time points: 0 s, 5 s and 10 s and hence can be included in future studies for healthy and dry eyes. Two OST indices (SD and RTD) were found not repeatable and the variability could be associated with the nature of these two indices. It was suggested that SD and RTD could be eliminated in future investigations.
4.7 References


5 Static and dynamic measurement of ocular surface temperature in dry eyes

Contributions
I designed this study in collaboration with my supervisors and co-authors. I was solely responsible for participant recruitment and data collection. I also analysed the data with useful guidance from my supervisors. I wrote the manuscript with helpful comments from my supervisors and co-authors.

Publications

Conference presentations
Part of the work in this paper was presented as an oral presentation at a scientific session of American Academy of Optometry (AAO) Nov 2013, Seattle, USA.

Acknowledgements
This study was funded by Singapore ToteBoard Organisation no. LS/CLS/TM/2009/007. The authors thank Dr. Cai Zhi Qiang from School of Electronic and Electrical Engineering in writing the OST analysis V2 program using MatLab Simulink 7.11.0 (R2010b) and Dr. Robert Straughan from School of Mathematics and Science for his valuable statistical advice.
5.1 Abstract

5.1.1 Purpose
To study ocular surface temperature (OST) in dry eyes by static and dynamic measures.

5.1.2 Methods
OST was recorded using NEC TH9260 thermo-tracer on 62 dry eye patients and 63 age- and sex-matched controls. Static measures were study of absolute OST at t = 0 s, 5 s and 10 s after eye opening. Dynamic measures were study of mean change and net change in OST over 10 s of sustained eye opening. Ten OST indices studied were: temperatures of the geometric center of the cornea (GCC), extreme temporal (T1) and nasal conjunctiva (T4), mid temporal (CT) and nasal conjunctiva (CN), temporal (LT) and nasal (LN) limbus, and mean (MOST), maximum (MaxT) and minimum (MinT) temperatures of the region of interest.

5.1.3 Results
For static measures, dry eye recorded a significantly lower GCC, MOST, MinT, MaxT, T4, CT, LT and LN at 0 s, 5 s and 10 s. The differences were highly significant (one-way ANOVA; p < 0.01) for GCC, MOST, MaxT, T4, CT and LT, significant (one-way ANOVA; p < 0.05) for MinT and LN at 0 s, 5 s and 10 s and marginal significant for CN at 5 s and 10 s. For dynamic measures, dry eye had significantly steeper regression line of mean change (corresponding to greater net change) for MaxT 5 s onward and T4 at 3 s onward.

5.1.4 Conclusions
Both static and dynamic measures of the OST were valuable and can be used as clinical tool to assess dry eye.

Keywords. dry eye, ocular surface temperature, ocular surface, static measures, dynamic measures
5.2 Introduction

Dry eye disease (DED) is multifactorial and can be caused by poor quality tear film and inflammation of the eyelid / ocular surface (Nichols et al., 2011) resulting from a lower tear production rate and / or a tear instability. In DED, the ocular surface becomes dry and lacks lubrication, eventually leading to ocular surface damage (DEWS, 2007). Infrared (IR) ocular thermography determines ocular surface temperature (OST) of the eye and pre-orbital skin by measuring the amount of IR radiation emitted from the surface with an infrared thermal imaging camera. Measurements are then processed into a color coded display image (thermogram) for interpretation and analysis (Morgan, 1993). Non-invasive ocular thermography was first introduced in 1968 and was used to evaluate both normal and pathological conditions (Mapstone, 1968a, Mapstone, 1968b, Mapstone, 1968c, Mapstone, 1968d).

Capturing OST changes using IR ocular thermography reflects the nature of the tear film and its stability (Craig et al., 2000, Labbe et al., 2007, Purslow and Wolffsohn, 2007) and has been used to study DED since 1993 (Morgan, 1993). Morgan and his associates (Morgan, 1993) investigated the temperature difference between limbus and central cornea (termed as RTD, radial temperature difference) on a 66 year old chronic dry eye patient and reported a significant difference in RTD between dry eye (1.40 °C) and healthy eye (0.37 °C). Further studies on dry eye have been performed using ocular thermometry / thermography since then (Morgan et al., 1993, 1995, 1996, Fujishima et al., 1996, Mori et al., 1997, Craig et al., 2000, Zelichowska et al., 2005, Singh and Bhinder, 2005a, Kamao et al., 2011, Su et al., 2011, Kottaiyan et al., 2012, Azharuddin et al., 2014, Zhang et al., 2015, Versura et al., 2015) and the findings are summarised in Table 5.1. Most of the reported studies only considered static measures (ie. study of OST on a single frame) or temporal / dynamic measures (ie. study of OST changes over time). The OST indices studied were limited and did not document the whole of the exposed ocular surface. Software used and method employed to precisely define anatomical locations across the ocular surface have also varied (Table 5.1). This study was designed to investigate the ocular surface temperature in dry eye using both static and dynamic measures. A novel ‘diamond’ method in marking the ocular surface and OST acquisition was employed Tan et al. (2016) and ten OST indices were included to document the whole inferior zone of the exposed ocular surface.
Table 5.1. Studies on dry eye using ocular thermography reported in the literatures.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects (mean age)</th>
<th>Thermo Tracer used (specification)</th>
<th>Ocular surface marking &amp; OST acquisition</th>
<th>OST indices studied</th>
<th>Static / dynamic (time upon eye opening)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morgan (1993)</td>
<td>1 D (66 yo)</td>
<td>IR Thermographer NEC6T62 (Sensitivity 0.1 °C Freq: 1 frame/s Resolution: 10x10 pixels)</td>
<td>-</td>
<td>RTD</td>
<td>Static</td>
</tr>
<tr>
<td>Morgan et al.</td>
<td>36 D (58yo)</td>
<td>IR thermographer NEC6T62 (Sensitivity 0.1 °C Freq: 1 frame/s Resolution: 10x10 pixels)</td>
<td>Five 10x10 pixel placed in 5 anatomical locations along horizontal meridian running across centre cornea</td>
<td>MOST RTD</td>
<td>Static</td>
</tr>
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<td>Morgan et al.</td>
<td>11 D (50yo)</td>
<td>IR thermographer NEC6T62 (Sensitivity 0.1 °C Freq: 1 frame/s Resolution: 10x10 pixels)</td>
<td>4 mm² area at the centre of cornea</td>
<td>GCC</td>
<td>Dynamic (7 s)</td>
</tr>
<tr>
<td>Fujishima et al.</td>
<td>20 D (37.9yo)</td>
<td>IR radiation thermometer THI-500 (Sensitivity: 0.1 °C Freq: 1 frame/s)</td>
<td>Central cornea</td>
<td>GCC</td>
<td>Static &amp; dynamic (10 s)</td>
</tr>
<tr>
<td>Mori et al. (1997)</td>
<td>13 D (45.5yo)</td>
<td>Thermal Vision Laird 3 (Nikon) (Sensitivity: 0.15 °C Freq: 60 frames/s)</td>
<td>20 x 20 pixel box at central cornea</td>
<td>K value</td>
<td>Dynamic (more than 10 s)</td>
</tr>
<tr>
<td>Craig et al. (2000)</td>
<td>8 &amp; 13 C (6.6yo)</td>
<td>IR thermographer NEC6T62 (Sensitivity 0.1 °C Freq: 1 frame/s Resolution: 10x10 pixels)</td>
<td>Mean of central cornea pixels</td>
<td>GCC TVF</td>
<td>Static</td>
</tr>
<tr>
<td>Zalcichowska et al.</td>
<td>9 D (33.6yo)</td>
<td>IR radiation thermographer</td>
<td>Central cornea</td>
<td>GCC</td>
<td>Static &amp; dynamic (15 s)</td>
</tr>
<tr>
<td>Singh and Bhinder</td>
<td>51 D (35.36yo)</td>
<td>IR and remote heat sensor thermometry (Sensitivity 0.1 °C)</td>
<td>Closed and open eye temperature for 5 s</td>
<td>MOST</td>
<td>Static</td>
</tr>
<tr>
<td>Kamao et al. (2011)</td>
<td>30 D (52.9yo)</td>
<td>Tomey IR thermographer (Sensitivity 0.1 °C Freq: 6 frame/s Resolution: 320 x 240 pixels)</td>
<td>Central cornea (GCC) 4 mm in diameter, CT and CN (both 2 mm in diameter)</td>
<td>GCC CN CT</td>
<td>Static &amp; dynamic (10 s)</td>
</tr>
<tr>
<td>Su et al. (2011)</td>
<td>47 C (34yo)</td>
<td>Microbolometer sensor (Sensitivity 0.1 °C Freq: 30 frame/s Resolution: 320 x 240 pixels)</td>
<td>ROI (region of interest) determined by four curves connected between four manually set apaxes (top and bottom of the eye, left and right corner of the eye)</td>
<td>TDV CV</td>
<td>Dynamic (6 s)</td>
</tr>
<tr>
<td>Kottaiyan et al.</td>
<td>51 C (35.36yo)</td>
<td>Thermovision A40 (Sensitivity 0.08 °C Freq: 30 frame/s Resolution: 320 x 240 pixels)</td>
<td>Central cornea</td>
<td>-</td>
<td>Dynamic (5 s)</td>
</tr>
<tr>
<td>Azharuddin et al.</td>
<td>42 D (35.3yo)</td>
<td>FLIR SC305 (Sensitivity &lt; 0.05 °C Freq: 9 frame/s Resolution: 320 x 240 pixels)</td>
<td>Cornea</td>
<td>-</td>
<td>Dynamic (15 s)</td>
</tr>
<tr>
<td>Zhang et al. (2015)</td>
<td>20 D (35.8yo)</td>
<td>FLIR SC325 (Sensitivity &lt; 0.05 °C Freq: 30 frame/s Resolution: 320 x 240 pixels)</td>
<td>Central cornea 7 ~ 9 mm circular</td>
<td>-</td>
<td>Dynamic (5 s)</td>
</tr>
<tr>
<td>Versura et al.</td>
<td>24 D (32 yo)</td>
<td>Tomey TG 1000</td>
<td>Central cornea 4 mm circular</td>
<td>CCT</td>
<td>Static</td>
</tr>
</tbody>
</table>

D – Dry eye subjects; C – Control subjects
RTD – Radial temperature difference; MOST – Mean ocular surface temperature; GCC – Geometrical centre cornea
TVF – Temperature variation factor; CN – Conjunctiva nasal; CT – Conjunctiva temporal
TDV – Temperature difference value (temporal variation of OST); CV – Compactness value (spatial variation of OST);
K value – steepening of corneal temperature
ADDE – Aqueous deficient dry eye; MGD – Meibomian gland dysfunction
CCT – Central corneal temperature
5.3 Methods

5.3.1 Subjects

The research protocol was approved by the Singapore National Health Group (NHG) Domain-Specific Review Board (DSRB) and the Singapore Polytechnic ethics review committee and the work adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from each subject at study enrolment. Sixty-two dry eye (48 ± 10 years; 14 Male and 48 Female) and 63 age- and sex-matched control subjects (46 ± 7 years; 16 Male and 47 Female) completed the study. The inclusion criteria for the dry eye subjects were as described previously (Tan et al., 2016): use of tear replacement therapy and had either a fluorescein tear break-up time of 10 seconds or less (Golding and Brennan, 1993), or a Schirmer I test result of less than 10 mm in 5 min (Morgan et al., 1995) along with presence of corneal or conjunctiva staining. All dry eye patients were screened and diagnosed by an ophthalmologist at Khoo Tech Puat Hospital eye clinic. Classification of mild or moderate and severe patients was based on a composite disease severity index, derived from the Dry Eye Workshop severity scale (DEWS, 2007a). Control subjects were those not using tear replacement therapy or any topical medication and without signs or symptoms of dry eye. All subjects were required to be noncontact lens wearers for at least two years prior to enrolment. Subjects were excluded from control group if they had Schirmer I test result of less than 10 mm in 5 min or fluorescein tear break-up time of 10 seconds or less. Subjects with any anterior ocular anomalies (e.g. current ocular infection, allergy or ptosis), those undergone surgery or taking any medication that could affect the tear film or who were currently pregnant or breastfeeding were excluded (Tan et al., 2016).

5.3.2 Procedures

The procedures were the same as described previously (Tan et al., 2016). Subjects were refrained from using their eye-drops or eye make-up on the day of measurement. Ocular thermography was performed in real time using an Infrared thermos tracer (NEC TH 9260) with resolution of 640 (H) x 480 (V) pixels, operational sensitivity of 0.06 °C and frequency of 30 frames per second, detecting infrared radiation between 8 and 14 µm. The emissivity of 0.98 was assumed (Mapstone, 1968d). A standard examination protocol as reported in the literatures (Morgan et al., 1995, Craig et al., 2000, Purslow and Wolffsohn, 2007, Kamao et al., 2011) was adopted. All the measurements were performed from 9 am to 2 pm in the same room with controlled room temperature (24.06 ± 0.41 °C) and humidity (49.76 ± 2.61 %), with no air drifts and same brightness (380 lux). Subjects were adapted to the room for 20 minutes prior to ocular thermography as previous work has shown that this period was necessary to achieve ocular temperature stabilisation (Morgan, 1994). As corneal temperature is strongly associated with body temperature and seemed to plateau at 36.5°C to 37.0°C (Kessel et al., 2010), body temperature for all subjects were measured and subjects with body temperature ≥ 37 °C were excluded. OST was recorded under the conditions described by Morgan and associates: the subjects blinked normally, closed for 3 s and the first image was recorded just after the eyes had opened (Morgan et al., 1995, Morgan et al., 1999). 0 s was recorded as the time upon eye opening. 300 frames of real time thermal images reflecting OST
changes at the ocular surface were captured over 10 s sustained eye opening. The measurement was done three times on right eye followed by left eye. At any time if subject blinked or changed fixation before 10 s, the measurement was discounted and repeated.

A novel ‘diamond’ method was used to mark the ocular surface using a custom-designed OST Analysis V2 software (developed using MatLab Simulink 7.11.0, R2010b). The region of interest (ROI) formed by five anatomical points across the ocular surface (labelled as 1 - 5) shaped like a diamond (Figure 5.1). This method has the advantages of (1) overcome reported problems of truncated image by upper lids (Pattmoller et al., 2014) and (2) minimize possible inconsistency in OST acquisition due to variation in palpebral aperture size and (3) enable study of the inferior zone of the ocular surface that reported to be a predictive area in detection of dry eye subtypes (Fenner and Tong, 2013). Each point marked represents an area of 3 × 3 pixels so that temperature was an average of nine pixels:-

1 - Temporal limbus (LT)
2 - Nasal limbus (LN)
3 - Extreme temporal conjunctiva (T1)
4 - Extreme nasal conjunctiva (T4)
5 - Most inferior point of the ocular surface

Once the marking of ROI was completed, OST acquisition and processing was performed automatically by double clicking the last point marked (point 5) to activate the OST Analysis V2 program and process all the 300 frames. All frame marking and data processing were undertaken by a single examiner (LL). Ten OST indices of the ocular surface were generated as shown in Figure 5.1.

Figure 5.1. Ocular surface marking and OST acquisition using the novel ‘diamond’ method.
Table 5.2. In this study, GCC denotes the temperature of the geometric center of the cornea, obtained midway between LT and LN. The OST indices were selected to document the whole inferior zone of the exposed ocular surface within ROI which had included most of the reported OST indices as shown in Table 5.1 with few newly added indices. All the ten OST indices extracted by the ‘diamond’ method has shown to be highly repeatable in assessing healthy and dry eyes (Tan et al., 2016) in terms of inter-image, inter-examiner and intra-examiner variability.

Table 5.2. The ten OST indices studied.

<table>
<thead>
<tr>
<th>OST indices</th>
<th>Description</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC</td>
<td>Geometric center of the cornea (midway between LT and LN)</td>
<td>Most commonly studied in the literature</td>
</tr>
<tr>
<td>MOST</td>
<td>Mean OST of the ROI</td>
<td>-</td>
</tr>
<tr>
<td>MinT</td>
<td>Minimum temperature of ROI</td>
<td>Study of the minimum and maximum temperature of the ocular surface</td>
</tr>
<tr>
<td>MaxT</td>
<td>Maximum temperature of ROI</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>Extreme temporal conjunctiva</td>
<td>Study of the different areas of the limbus and conjunctiva</td>
</tr>
<tr>
<td>T4</td>
<td>Extreme nasal conjunctiva</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Mid-temporal conjunctiva (midway between T1 and LT)</td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>Temporal limbus</td>
<td></td>
</tr>
<tr>
<td>LN</td>
<td>Nasal limbus</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>Mid-nasal conjunctiva (midway between T4 and LN)</td>
<td></td>
</tr>
</tbody>
</table>

5.3.3 Data analysis

Static measures were study of absolute OST at t = 0 s, 5 s and 10 s after eye opening. Dynamic measures were study of mean change (relative to baseline) as well as net change in OST over 10 s of sustained eye opening. To prevent difficulties arising when non-independent data were collected from both eyes, only data obtained from right eye were used in the analysis (Ray and O'Day, 1985). Statistical analyses were performed using JMP version 12.1.0 (http://www.jmp.com; SAS Institute Inc., USA). For static measures, one-way ANOVA was performed for the ten indices studied to explore differences between dry eye and control subjects at t = 0 s, 5 s, and 10 s. For dynamic measures, a general linear model was constructed (two-way repeated measures ANOVA model) to test the significance of each group, time and their interaction (group by time) effects, where the interaction between groups over time was the key outcomes. Post-hoc analysis for significance of the group effect at each time point was conducted using unpaired t-test (two-tailed), on OST indices with significant outcomes. All tests were two-tailed and p < 0.05 was considered significant. All data were presented as mean ± SD, unless otherwise stated.

5.4 Results

All dry eye subjects were mild to moderate with no inflamed meibomian glands. It was acknowledged that many disease severity criteria are confounded by complex disease subtypes and a lack of standardisation, and the selection of single criteria for assessment of disease severity is therefore fraught with difficulties (Schein et al., 1997, DEWS, 2007b, Sullivan et al., 2010). Both
dry eye and control subjects showed positive compliance during ocular thermography measurements and therefore it was possible to record reliable data in all cases. No reports of reflex tearing and any discomfort from the subjects during the course of the study. Although eight seconds of sustained eye opening has been reported to be an easily achievable target for subjects without causing reflex tearing (Purslow and Wolffsohn, 2005), other studies had reported to be equally achievable for subjects to hold for 10 s (Mori et al., 1997, Kamao et al., 2011).

For static measures, dry eye recorded a significantly lower temperature (for GCC, MOST, MinT, MaxT, T4, CT, LT and LN) as compared to controls at 0 s, 5 s and 10 s (one-way ANOVA, p < 0.05). The differences were highly significant (p < 0.01) for GCC, MOST, MaxT, T4, CT and LT and were significant (p < 0.05) for MinT and LN. There were marginal significant differences found for CN at 5 s and 10 s and no significant differences found between the two groups for T1 (Figure 5.2).

For dynamic measures, dry eye had significantly steeper regression line of mean change (corresponding to greater net change) only for two out of the ten OST indices: MaxT and T4 (Figure 5.3). Two-way analysis of variance showed that there were significant group by time interaction effects for MaxT and T4 temperatures (MaxT: F = 4.6814, p = 0.0324; T4: F = 5.9506, p = 0.0161). For MaxT, the drop in mean change in dry eye was statistically significant from 5 s onward (unpaired t-test, 5 s, p = 0.037; 6 s, p = 0.012; 7 s, p = 0.022; 8 s, p = 0.023; 9 s, p = 0.016; 10 s, p = 0.019). Net change for MaxT in dry eye over 10 s was -0.17 ± 0.17 °C, which was two times greater as compared to controls (-0.09 ± 0.21 °C). Cooling rate as indicated by gradient of the graph for MaxT was also twice as much as in dry eye (-0.0164 °C/s) as compared to controls (-0.0072 °C/s) (Figure 3). For T4, the drop in mean change was only happened in dry eye group and it was statistically significant from 3 s onward (unpaired t-test, 3 s, p = 0.023; 4 s, p = 0.005; 5 s, p = 0.014; 6 s, p = 0.009; 7 s, p = 0.047; 8 s, p = 0.036; 9 s, p = 0.028; 10 s, p = 0.005). T4 for control group was pretty stable during the 10 s of sustained eye opening. Net change for T4 in dry eye over 10 s was -0.09 ± 0.22 °C, which was more than two times greater as compared to controls (-0.04 ± 0.24 °C). Cooling rate for T4 was also more than two times greater in dry eye (-0.0091 °C/s) as compared to controls (-0.0039 °C/s). No significant differences were found in mean change at any point of time (unpaired t-test at each 1 s interval, p > 0.05) between the two groups for other OST indices during dynamic measures (Figure 5.3).
Figure 5.2. *(Static measures)* Box plots showing comparison of absolute OST at 0 s, 5 s and 10 s: A. GCC; B. MOST; C. MinT; D. MaxT; E. T1; F. T4; G. CT; H. LT; I. LN and J. CN in (grey box) dry eye subjects and (white box) controls. The results were expressed as median and mean ± SD. Mean-connecting-lines are represented by dotted lines to show the change in mean over 0 s, 5 s and 10 s. *p values* are shown using one-way ANOVA at 95% CI.
Figure 5.3. (Dynamic measures) Graphs showing the mean change OST (relative to baseline) during the 10 s sustained eye opening: A. GCC and MOST; B. MinT & MaxT; C. T1; D. T4; E. CT & LT; F. CN & LN in (solid circles) dry eye subjects and (open circles) controls. Values in boxes represent the cooling rate and net change in OST over the 10 s period in dry eye and control groups respectively. A comparison of mean at each 1 s interval was performed using unpaired t-test, *p < 0.05. A typical standard deviation for GCC was ± 0.29 (= average std deviation) for dry eye and ± 0.30 for controls and for MOST was ± 0.15 for dry eye and ± 0.18 for controls. A typical standard deviation for MinT was ± 0.33 for dry eye and ± 0.32 for controls and for MaxT was ± 0.11 for dry eye and ± 0.13 for controls. A typical standard deviation for T1 was ± 0.14 for dry eye and ± 0.11 for controls and for T4 was ± 0.21 for dry eye and ± 0.16 for controls. A typical standard deviation for CT was ± 0.19 for dry eye and ± 0.17 for controls and for LT was ± 0.23 for dry eye and ± 0.29 for controls. A typical standard deviation for CN was ± 0.14 for dry eye and ± 0.16 for controls and for LN was ± 0.22 for dry eye and ± 0.23 for controls.
5.5 Discussion

The current study demonstrated the ability of IR ocular thermography in assessing dry eye. Ten OST indices were evaluated in two aspects: static and dynamic measures. Each OST index studied represented area of 3 x 3 pixels, except for MOST. Rather than report on individual pixel values which might be subject to local variation, 3 x 3 pixels was selected and the temperature averaged. This is a good compromise between single pixels and a larger ROI which would provide less opportunity to analyse specific geographic areas of interest.

OST in dry eye was different from controls at different ocular surface areas during static measures upon eye opening (t = 0 s) as well as when t = 5 s and 10 s. As compared to controls, the ocular surface of dry eye subjects was significantly cooler as recorded at the geometric center of the cornea (GCC) as well as various areas at conjunctiva (T4, CT and CN) and limbus (LT and LN) and causing an overall lower mean ocular surface temperature (MOST). As the ocular surface measured by the thermo-tracer consist of cornea-conjunctiva-limbal complex, it was not surprise to record a significantly lower minimum temperature of the ocular surface (MinT) and maximum temperature of the ocular surface (MaxT) in dry eye. During dynamic measures, OST was found to drop over the 10 s of sustained eye opening in both the dry eye and control subjects. Only two OST indices (out of ten) had significant steeper regression line of mean change with greater net change in dry eye. As for the temperature of the extreme nasal conjunctiva (T4), the change was only observed in dry eye subjects and was statistically significant from 3 s onward.

Static measures

The primary source of ocular radiation measured by ocular thermography is the tear film (Morgan et al., 1995) so changes in tear film thickness and its composition alter the temperature measured (Craig et al., 2000). Lower OST in the dry eye group found in the current study could be due to a thinner tear film as a result of a thinner tear film lipid layer (TFLL) in dry eye (Nichols et al., 2005, King-Smith et al., 2013). TFLL has been reported to be important in tear film stability and evaporation (Craig and Tomlinson, 1997) and is abnormal in dry eye (Craig et al., 2000, Doane, 1994). According to the Dry Eye Workshop report (DEWS, 2007a), tear film instability is one of the two core mechanisms of dry eye and can leads to thickness variation and an overall thinner tear film. The ocular surface was cooled by a thinner tear film leading to lower temperature recorded on various ocular surface areas (geometric centre of the cornea, conjunctiva and limbus) during static measures at t = 0 s. OST was likely to be affected by variations in evaporation in the seconds after eye opening, in addition to the effects of convection (Craig et al., 2000). Not unexpectedly, lower temperatures were noted in dry eye subjects after 5 s and 10 s of eye opening. Thermography has been reported as an indirect method to evaluate tear evaporation rates and tear film impairment due to its ability to record subtle changes over the corneal temperature (Mori et al., 1997). Indeed, tear evaporation in dry eye can cause a 10-fold reduction in tear thickness after a blink (Nichols et al., 2012) and found to be correlated with lower corneal temperature and subjective discomfort symptoms (Versura et al., 2015). This was apparent in the current report revealing a declining
temperature on the various ocular surface areas from 0 s to 10 s during static measures. The temperature gradient varying at different OST indices and suggestive of different evaporation rate at different areas of the ocular surface. Lower OST can also be accounted for by the presence of “cold receptors,” a class of ion channels identified in nerve endings and in corneal and conjunctival epithelial cells that can mediate the pain transduction from the ocular surface (Belmonte and Gallar, 2011). Although temperature variation has been reported to be higher in dry eyes when RTD (radial temperature difference) was studied by Morgan et al. (1993,1995), RTD was not included in the current study as previous report (Tan et al., 2016) has shown poor RTD repeatability when measured using the current thermo tracer.

A lower MOST values in dry eye was in agreement with the report by (Singh and Bhinder, 2003, 2005a) using remote sensor thermometry but conflicts with the report by Morgan et al. (1995) and Singh and Bhinder (2005b) using IR thermography and IR thermometry respectively. A warmer overall ocular surface has been accounted for by the increased conjunctival hyperaemia in dry eye (Morgan et al., 1995, Singh and Bhinder, 2005b). The vascularised conjunctiva is an important heat source (Morgan, 1993) to the ocular surface. Certainly, OST is increased during inflammatory disease (Mapstone, 1968b, Morgan, 1993) and ocular surface inflammation is a core mechanism in dry eye (Yi and Asbell, 2014). A higher MOST could also be associated to higher blink rate in dry eye (Tan et al., 2009). The conflicting results found in this study as compared to those previously reported may be due to various reasons. Firstly, most of the dry eye subjects did not present with conjunctival hyperaemia, the results were therefore different. Secondly, different experimental methodologies may lead to different findings as suggested by Kamao et al. (2011). The ‘diamond’ method in marking the ocular surface and OST acquisition in this study allowed a more holistic study on MOST as it covers a wider area of the ocular surface as compared to obtaining the MOST by averaging the temperature of the cornea and conjunctiva across the horizontal meridian in (Morgan et al., 1995)’s study. Furthermore, the ROI studied was the lower half of the ocular surface whereby tear film will thin faster (Fenner and Tong, 2013) as compared to other areas due to evaporation and leads to a lower MOST in dry eye as shown in the current study. OST measurements by Singh and Bhinder (2005b) were made in a closed chamber instead of an open atmosphere so comparison with that work is clearly problematic as local environmental factors influence OST (Freeman and Fatt, 1973). Thirdly, the severity of dry eye varied by report. Dry eye subjects recruited by (Morgan et al., 1995) were mostly severe dry eye whereas the dry eye subjects in this study ranged from mild to moderate cases. In more severe cases, the level of local inflammation and greater conjunctiva hyperaemia may overwhelm evaporative effects, leading to a warmer ocular surface (Morgan et al., 1995). Last but not least, age of subjects recruited could also cause different in results. In previous projects, there was no apparent attempt to age-match the dry eye and the control groups. This is important because OST was reported to decrease with age at a rate of - 0.010 °C / year (Morgan, 1999) and the dry eye populations used by previous reports (Morgan et al., 1995, Mori et al., 1997, Kamao et al., 2011, Su et al., 2011) were much older.
compared to the controls; in other words, differences between the groups may be age-related rather than to the disease itself.

Dynamic measures
During dynamic measures, results were in agreement with literatures that ocular surface cooled during sustained eye opening (Morgan et al., 1996, Li et al., 2015) and rate of the cooling was greater in the dry eye group (Morgan et al., 1996). This was also in consistent with a mathematical model developed by Peng et al. (2014) which postulates a mechanism by which local rupture of the TFLL increases local tear evaporation rate leading to tear film rupture and tear film break-up. Tear film thinning and break-up has shown to correspond with ocular surface cooling over time (Li et al., 2015). Dry eye had a thinner TFLL (Nichols et al., 2005, King-Smith et al., 2013) and upon eye opening, the tear film starts to thin due to evaporation leading to drop in temperature (Craig and Tomlinson, 1997). The tear film lipid could have depleted and no longer hold / protect the aqueous layer at $t = 5$ s (for MaxT) and at $t = 3$ s (for T4) and hence a sudden increase in evaporation / tear film thinning and ocular surface cooling. The cooling rate for MaxT was twice as much as in dry eye as compared to controls and may indirectly reflect the rate of evaporation in dry eyes as reported by Li et al. (2015). A two-fold (Rolando et al., 1983) and a three-fold (Mathers et al., 1993) increase in the tear evaporation rate in dry eyes has been reported previously. Although tear evaporation was not measured in the current study, tear film cooling in dry eye upon eye opening as a result of tear evaporation as well as greater effect of the positive latent heat of tear vaporisation has also been reported (Sears et al., 1991, Morgan et al., 1996, Craig et al., 2000).

Changes in T4 was only observed in dry eye subjects and could be easily differentiated from their controls (Figure 5.3). Conjunctival temperature was reported to be higher than the central cornea (Mapstone, 1968d, Alio and Padron, 1982). Although the reasons remains unclear, temperature of the nasal conjunctiva was reported to be higher than that of the temporal conjunctiva because of the influence of greater blood flow due to more large vessels including the dorsal nasal artery and the angular artery at the nasal conjunctiva (Kamao et al., 2011). There are more large vessels, including the dorsal nasal artery and the angular artery, on the nasal side of the eye, and the medial rectus muscle has two anterior ciliary arteries, whereas the lateral rectus muscle has only one artery (Kamao et al., 2011). The difference in vascularisation at nasal and temporal conjunctiva could have created different tear film cooling rate in these two areas upon eye opening and therefore the different results found in T4 and T1. The reason why there was no change in T4 for the controls warranted further investigations. Studying T4 at 3 s and onwards can be a potential diagnostic index for dry eye due to its ‘unique’ behaviour as compared to non-dry eye subjects. Based on the findings, 10 s of sustained eye opening may not be required as it is hard for dry eye patients to keep their eyes open for 10 s without inducing reflex tearing and blinking.
5.6 Conclusion

Static and dynamic measurement of the OST provided two different aspects in studying the tear film. Both measurements were useful and can be used as clinical tool to assess dry eye.

5.7 References


Li W, Graham AD, Selvin S, Lin MC. Ocular Surface Cooling Corresponds to Tear Film Thinning and Breakup. Optom Vis Sci 2015; 92(9): e248-56.


6 Screening for dry eye disease using infrared ocular thermography

Contributions
I designed this study in collaboration with my supervisors and co-authors. I was solely responsible for participant recruitment and data collection. I also analysed the data with useful guidance from my supervisors. I wrote the manuscript with helpful comments from my supervisors and co-authors.

Publication

Conference presentations
BCLA-Asia, 13-14th September 2016, Cordis Langham Place, Hong Kong (paper accepted for oral presentation).

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6.1 Abstract

6.1.1 Purpose
To evaluate the efficacy of infrared (IR) ocular thermography in screening for dry eye disease (DED).

6.1.2 Methods
IR ocular thermography was performed on 62 dry eye and 63 age- and sex-matched control subjects. Marking of ocular surface and temperature acquisition was done using a novel ‘diamond’ demarcation method. 30 static- and 30 dynamic-metrics were studied and receiver operating characteristic curves were plotted. Efficacy of the temperature metrics in detecting DED were evaluated singly and in combination in terms of their area under the curve (AUC), Youden’s index and discrimination power (DP).

6.1.3 Results
Absolute temperature of the extreme nasal conjunctiva 5 s and 10 s after eye opening were best detectors for DED. With threshold value for the first metric set at 34.7 °C, sensitivity and specificity was 87.1% (95% CI: 76.2 to 94.3%) and 50.8% (95% CI: 37.9 to 63.6%) respectively. With threshold value for the second metric set at 34.5 °C, sensitivity and specificity was 77.6% (95% CI: 64.7 to 87.5%) and 61.9% (95% CI: 48.8 to 73.9%) respectively. The two metrics had moderate accuracy and limited performances with AUC of 72% (95% CI: 63 to 81%) and 73% (95% CI: 64 to 82%); Youden index of about 0.4 and DP of 1.07 and 1.05 respectively. None of the dynamic metrics was good detector for DED. Combining metrics was not able to increase the AUC.

6.1.4 Conclusions
This work suggests some utility for the application of IR ocular thermography for evaluation of dry eye patients.

Keywords. Dry eye disease, IR ocular thermography, ocular surface, static metrics, dynamic metrics
6.2 Introduction

Dry eye disease (DED) is a commonly encountered condition in clinical practice and affects up to 12.3% of the population in Singapore (Tan et al., 2015) with a world prevalence range of 5% to 38% (DEWS, 2007a). The condition has remarkable impact on daily social and physical functioning, workplace productivity and quality-of-life (Miljanovic et al., 2004, Miljanovic et al., 2007, Pflugfelder, 2008, Tong et al., 2010). Diagnosing the disease can be a tedious and challenging task (Savini et al., 2008) and been hampered by the lack of objective tests with sufficient sensitivity and specificity, adequate repeatability, ease of performance, and suitability for the clinical practice setting particularly in early or mild cases (DEWS, 2007b). Due to its multifactorial nature, DED potentially requires a broad spectrum of test measures in the monitoring of its diagnosis and treatment (Tomlinson et al., 2013). While there are many clinical tests for DED, the diagnostic values can be inconclusive (Goren and Goren, 1988, Kallarackal et al., 2002) and may not be repeatable and/or reliable because of variable results, poor reproducibility and low sensitivity (Saleh et al., 2006, Cho et al., 1992; Cho et al., 1993a; Cho et al., 1993b). Determining the cause of dry eye when minimal clinical signs are present is difficult and the diagnosis is complicated further when there is a lack of correlation between its signs and symptoms (Nelson et al., 2000, Kallarackal et al., 2002, Begley et al., 2003, Nichols et al., 2004b, Moore et al., 2009, Cardona et al., 2010, Lemp et al., 2011, Sullivan et al., 2014).

Tear film stability is a key test in screening and diagnosing DED (DEWS, 2007b). It has been reported that capturing ocular surface temperature (OST) changes using infrared (IR) ocular thermography reflects the nature of the tear film (Craig et al., 2000, Labbe et al., 2007) and its stability (Purslow and Wolffsohn, 2007) and can be used to screen DED (Kamao et al., 2011, Su et al., 2011). However, temperature metrics available from ocular thermography to screen DED were limited and remain unclear. Most studies on OST and dry eye have evaluated the temperature of the geometric center of the cornea (Fujishima et al., 1996, Morgan et al., 1996, Craig et al., 2000, Zelichowska et al., 2005, Kamao et al., 2011). A small number of studies evaluated other metrics such as the relative differences in temperature across the ocular surface (Morgan et al., 1993, Morgan et al., 1995) mean ocular surface temperature (Morgan et al., 1995, Singh and Bhinder, 2005a), temperature at the nasal and temporal conjunctiva (Kamao et al., 2011) and temperature difference and compactness values of the OST (Su et al., 2011). Two reports on dry eye screening using ocular thermography were done using temperature of the geometric center of the cornea (Kamao et al., 2011) and temperature difference and compactness values of the OST (Su et al., 2011).

The current study was devised to evaluate the efficacy of IR ocular thermography as a diagnostic tool for DED and to determine the most effective temperature metrics, applied singly or in combination.
6.3 Methods

6.3.1 Subjects

The research protocol was approved by the Singapore National Health Group (NHG) Domain-Specific Review Board (DSRB) and the Singapore Polytechnic ethics review committee and the work adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from each subject at study enrolment. Sixty-two dry eye (48 ± 10 years; 14 Male and 48 Female) and 63 age- and sex-matched control subjects (46 ± 7 years; 16 Male and 47 Female) completed the study with the age matching range of ± 2 years. This study included mild or moderate dry eye subjects. They were classified based upon a composite disease severity index, derived from the Dry Eye Workshop severity scale (DEWS, 2007c). The inclusion criteria for the dry eye subjects were as described previously (Tan et al., 2016): use of tear replacement therapy and had either a fluorescein tear break-up time of 10 seconds or less (Golding and Brennan, 1993), or a Schirmer I test result of less than 10 mm in 5 min (Morgan et al., 1995) along with presence of corneal or conjunctival staining. All dry eye patients were screened and diagnosed by an ophthalmologist at Khoo Tech Puat Hospital eye clinic. Control subjects were those not using tear replacement therapy or any topical medication and without signs or symptoms of dry eye. All subjects were required to be noncontact lens wearers for at least two years prior to enrolment. Subjects were excluded from control group if they had Schirmer I test result of less than 10 mm in 5 min or fluorescein tear break-up time of 10 seconds or less. Subjects with any anterior ocular anomalies (e.g. current ocular infection, allergy or ptosis), those undergone surgery or taking any medication that could affect the tear film or who were currently pregnant or breastfeeding were also excluded.

6.3.2 Procedures

The procedures were the same as described previously (Tan et al., 2016). Subjects were asked to refrain from using their eye-drops or eye make-up on the day of measurement. Ocular thermography was performed in real time using an Infrared thermo tracer (NEC TH 9260) with resolution of 640 (H) x 480 (V) pixels, operational sensitivity of 0.06 °C and frequency of 30 frames per second, detecting infrared radiation between 8 and 14 µm. The emissivity of 0.98 was assumed (Mapstone R, 1968d). A standard examination protocol as reported in the literatures (Morgan et al., 1995, Craig et al., 2000, Purslow and Wolffsohn, 2007, Kamao et al., 2011) was adopted. All the measurements were performed from 9 am to 2 pm in the same room with controlled room temperature (24.06 ± 0.41 °C) and humidity (49.76 ± 2.61 %), with no air drifts and same brightness (380 lux). Subjects were adapted to the room for 20 minutes prior to ocular thermography as previous work has shown that this period was necessary to achieve ocular temperature stabilisation (Morgan, 1994). OST was recorded under the conditions described by Morgan and associates: the subjects blinked normally, closed for 3 s and the first image was recorded just after the eyes had opened (Morgan et al., 1995, Morgan et al., 1999a). 0 s was recorded as the time upon eye opening. 300 frames of real time thermal images reflecting OST changes at the ocular surface were captured over 10 s sustained eye opening. The measurement
was done three times on right eye followed by left eye. At any time if subject blinked or changed fixation before 10 s, the measurement was discounted and repeated.

A novel ‘diamond’ method was used to mark the ocular surface using a custom-designed OST Analysis V2 software (developed using MatLab Simulink 7.11.0, R2010b). The region of interest (ROI) formed by five anatomical points (labelled as 1 - 5) shaped like a diamond (Fig. 6.1). This method has the advantages of (1) overcome problems of truncated image by upper lids (Pattmoller et al., 2014) and (2) minimize possible inconsistency in OST acquisition due to variation in palpebral aperture size and (3) enable study of the inferior zone of the ocular surface that reported to be a predictive area in detection of dry eye subtypes (Fenner and Tong, 2013). Each point marked represents an area of 3 × 3 pixels so that temperature was an average of nine pixels:

1 - Temporal limbus (LT)
2 - Nasal limbus (LN)
3 - Extreme temporal conjunctiva (T1)
4 - Extreme nasal conjunctiva (T4)
5 - Most inferior point of the ocular surface

![Figure 6.1. The ‘diamond’ method in marking the ocular surface and OST acquisition. ROI = region of interest.](image)

Once the marking of ROI was completed, OST acquisition and processing was performed automatically by double clicking the last point marked (point 5) to activate the OST Analysis V2 program and process all the 300 frames. All frame marking and data processing were undertaken by a single examiner (LL). Ten OST indices of the ocular surface were generated as shown in Table 6.1. In this study, GCC denotes the temperature of the geometric center of the cornea, obtained midway between LT and LN. The OST indices were selected to document the whole inferior zone of the exposed ocular surface within ROI and to include as far as possible, all the reported temperature metrics (Morgan et al., 1993, 1995, 1996, Fujishima et al., 1996, Craig et al., 2000, Zelichowska et al., 2005, Singh and Bhinder, 2005a, Kamao et al., 2011, Su et al., 2011). All
the ten OST indices extracted by the ‘diamond’ method has shown to be highly repeatable in assessing healthy and dry eyes (Tan et al., 2016) in terms of inter-image, inter-examiner and intra-examiner variability.

Table 6.1. The ten OST indices studied.

<table>
<thead>
<tr>
<th>OST indices</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC</td>
<td>Geometric center of the cornea (midway between LT and LN)</td>
</tr>
<tr>
<td>MOST</td>
<td>Mean OST of the ROI</td>
</tr>
<tr>
<td>T1</td>
<td>Extreme temporal conjunctiva</td>
</tr>
<tr>
<td>T4</td>
<td>Extreme nasal conjunctiva</td>
</tr>
<tr>
<td>CT</td>
<td>Mid-temporal conjunctiva (midway between T1 and LT)</td>
</tr>
<tr>
<td>LT</td>
<td>Temporal limbus</td>
</tr>
<tr>
<td>LN</td>
<td>Nasal limbus</td>
</tr>
<tr>
<td>CN</td>
<td>Mid-nasal conjunctiva (midway between T4 and LN)</td>
</tr>
<tr>
<td>MinT</td>
<td>Minimum temperature of ROI</td>
</tr>
<tr>
<td>MaxT</td>
<td>Maximum temperature of ROI</td>
</tr>
</tbody>
</table>

6.3.3 Data Analysis

Data on all 62 dry eye subjects and 63 controls were tabulated and analysed. The ten OST indices were studied in two aspects: static and dynamic measures. To prevent difficulties arising when non-independent data were collected from both eyes, only data obtained from right eye were used in the analysis (Ray and O'Day, 1985).

Static measures were study of absolute OST at \( t = 0 \) s, \( 5 \) s and \( 10 \) s (3 static attributes) after eye opening. Data were obtained directly from the raw data. As there were ten OST indices, 30 static temperature metrics were generated. For example, GCC temperature at \( 0 \) s, \( 5 \) s and \( 10 \) s were labelled as GCC-0, GCC-5 and GCC-10 respectively.

Dynamic measures were study of temperature change over time. One phase exponential curve was fitted to the temperature vs. time data for each series of images using JMP 11 according to the following model:

\[
\text{Temperature} = a + b \times \text{Exp}(c \times \text{time}),
\]

The terminology used by JMP (http://www.jmp.com; SAS Institute Inc., USA) such as ‘asymptote’ (a), ‘scale’ (b) and ‘growth rate’ * [3 dynamic attributes] (c) were adopted. Again, as there were ten OST indices, in total 30 dynamic temperature metrics were generated. For example, asymptote, scale and growth rate for GCC temperature were labelled as GCC-A, GCC-S and GCC-GR respectively. The efficacy of ocular thermography in diagnosing DED was then evaluated in two
phases: singly and in combination.

**Phase 1: Evaluating the efficacy of the 30 static- and 30 dynamic-metrics when applied singly**

Findings on dry eye subjects and their controls for each metric were compiled. Using GraphPrism 6 ([www.graphpad.com](http://www.graphpad.com); GraphPad Software Inc., USA), a range of testing threshold/criterion with their sensitivity, specificity, area under the ROC curve (AUC), predictive values (Bland and Altman, 1994a, 1994b) were then been derived. Receiver operating characteristics (ROC) (Zweig and Campbell, 1993, Bland and Altman, 1994c) curves, which determine the sensitivity and specificity of the measurement in diagnosing dry eye (Khanal et al., 2008) were plotted for all 30 static and 30 dynamic-metrics and AUC was extracted using trapezoidal numerical integration. The AUC (range: 50 to 100 in percentage) is a quantitative representation of overall test accuracy, where values from 50 to 70 represent low accuracy, values from 70 to 90 represent tests that are useful for some purposes/moderate accuracy, and values > 90 represent tests with high accuracy (Wians FH, 2009). Metrics that has AUC of 70 or above were selected.

Although AUC is one of the main parameters of ROC, to better evaluate the performance of the technique as a detector, (i.e. the discrimination between dry eyes from those of normal subjects) it is important to determine the cutoff values, selected as the one that optimizes both sensitivity and specificity (Bland and Altman, 1994a, Alonso-Caneiro et al., 2011). Sensitivity is the proportion of actual positives (i.e. dry eye subjects) that are correctly identified, while specificity is the proportion of actual negatives (i.e. normal subjects) that are correctly identified. To facilitate the detector performance comparison, every tested metric by means of a set of statistical tools (Sokolova et al., 2006) was evaluated, namely the Youden’s index ($Y$) (Youden, 1950) and the discrimination power (DP) (Sokolova et al., 2006).

Youden’s index evaluates the algorithm’s ability to avoid failure and follows the expression

$$Y = \text{sensitivity} + \text{specificity} - 100.$$

Its value ranges from 0 to 100 in percentage, and has a zero value when a diagnostic test gives the same proportion of positive results for groups with and without the disease, i.e. the test is useless. A value of 100 indicates that there are no false positives or false negatives, i.e. the test is perfect. Youden’s index is often used in conjunction with ROC analysis (Schisterman et al., 2005).

Graphically, $Y$ is the maximum vertical distance between the ROC curve and the diagonal line. Cutoff values of the selected metrics can then be determined as the criterion that maximized the Youden index: max (sensitivity$_c$ + specificity$_c$ – 100), where $c$ ranges over all possible criterion values (Youden, 1950). The cutoff values that achieves this maximum is referred to as the optimal cutoff because it is the cutoff values that optimizes the metric’s differentiating ability when equal weight is given to sensitivity and specificity (Bland and Altman, 1994a, Alonso-Caneiro et al., 2011, Schisterman et al., 2005, Faraggi D, 2000; Reiser B, 2000).
DP is a measurement that summarizes sensitivity and specificity of the technique,

\[ DP = \sqrt[3]{\frac{3}{\pi}} \log \left( \frac{X}{100 - X} \right) \]

Where \( X = \text{sensitivity} / (100 - \text{sensitivity}) \) and \( Y = \text{specificity} / (100 - \text{specificity}) \). Values of \( DP < 1 \) indicate poor discrimination performance, \( DP < 2 \) indicates limited performance, \( DP < 3 \) considered to be a fair discrimination, while values above 3 are classified as good.

Calculating the Youden’s index and DP could give clearer evidence of a test performance (Alonso-Caneiro et al., 2011). In this report, AUC was used as an indicator for test accuracy (Wians, 2009) and where the AUC does not show much difference, Youden’s index and DP were used as indicators for test performance (Alonso-Caneiro et al., 2011).

**Phase 2: Evaluating the best combined temperature metrics in screening DED**

The diagnostic power of the metrics can be possibly maximized by combining them. In this part of the study, AUC was evaluated if it can be further maximised by a factor analysis model using principal component analysis (Kass and Tinsley, 1979, Everitt and Dunn, 1991) through Excel’s ‘solver’ function (Microsoft Excel 2013, USA). The analysis was developed for each dataset in order to reduce the dimensionality of the variables down to one or two factors combining these variables. This will help to determine the best detectors for dry eye. Again, data was first tabulated on the 30 static- and dynamic-metrics. A range of testing threshold/criterion was encapsulated in 0.1 intervals. Using Excel spreadsheet, sensitivity, specificity and AUC were derived for all the metrics. After which, a tool in the Excel spreadsheet ‘solver’ was used to test on all possible combinations within the 30 static- and dynamic-metrics that could possibly maximising the AUC and determine the best detectors.

**6.4 Results**

**Phase 1: Evaluating the efficacy of the 30 static- and 30 dynamic-metrics when applied singly**

Figure 6.2 shows the ROC curves for the 30 static metrics, grouped in three different time points (0 s, 5 s and 10 s). Figure 3 shows the ROC curves for the 30 dynamic metrics, grouped into its three attributes.

Based on Figure 6.2, the best AUC for static metrics were T4-5 and T4-10 with AUC of 72% (95% CI: 63 to 81%) and 73% (64 to 82%) respectively, indicating the tests were useful for some purpose/moderate accuracy (Wians, 2009). AUC for the rest of the static metrics lied below 70% indicating low accuracy. For example, AUC for T4-0 was 69% (60 to 79%). Based on Figure 6.3, the AUC for dynamic metrics ranged from 50 to 64, indicating that all dynamic metrics were of low accuracy as their AUC lied below 70% (Wians, 2009).
Figure 6.2. ROC curves for static metrics at three different time points. AUC are shown at the legend.
Figure 6.3. ROC curves for dynamic metrics at three different attributes. AUC are shown at the legend.
Table 6.2 shows a summary of AUC, sensitivity, specificity, Youden's index, DP and the selected cutoff values for the 30 static metrics. The best results were, again, obtained for T4-5 and T4-10 metrics with DP of 1.07 and 1.05 respectively indicating limited performance. DP for the rest of the static metrics were less than 1 indicating poor discrimination performance including T4-0 (Sokolova et al., 2006). Youden’s index for T4-5 and T4-10 was also found to be highest of all (37.9 and 39.5 respectively). T4-0 had lower DP of 0.79 and Youden’s index of 34.4.

When the cutoff values for T4-5 was set at 34.7 °C (i.e., values < 34.8 °C; Table 6.2), sensitivity and specificity was 87.1% (76.2 to 94.3%) and 50.8% (37.9 to 63.6%) respectively and when the
cutoff values for T4-10 was set at 34.5 °C (i.e., values < 34.6 °C; Table 6.2), sensitivity and specificity was 77.6% (64.7 to 87.5%) and 61.9% (48.8 to 73.9%) respectively.

Table 6.3. Test effectiveness for the 30 dynamic metrics (A-asymptote, S-scale, GR-growth rate). AUC, sensitivity, specificity, Youden’s index (Y), discrimination power (DP) and the selected cutoff values are shown.

<table>
<thead>
<tr>
<th>Dynamic metrics-A</th>
<th>AUC, % (95% CI)</th>
<th>Cutoff values, °C</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>Y</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC-A</td>
<td>51 (41 to 61)</td>
<td>&gt; -0.2861</td>
<td>40.3 (28.1 to 53.6)</td>
<td>68.3 (55.3 to 79.4)</td>
<td>8.6</td>
<td>0.21</td>
</tr>
<tr>
<td>MOST-A</td>
<td>56 (46 to 66)</td>
<td>&lt; -0.5155</td>
<td>45.2 (32.5 to 58.3)</td>
<td>73.0 (60.4 to 83.4)</td>
<td>18.2</td>
<td>0.44</td>
</tr>
<tr>
<td>MinT-A</td>
<td>54 (44 to 64)</td>
<td>&lt; -0.2918</td>
<td>71.0 (58.1 to 81.8)</td>
<td>42.9 (30.5 to 56.0)</td>
<td>13.8</td>
<td>0.33</td>
</tr>
<tr>
<td>MaxT-A</td>
<td>62 (52 to 72)</td>
<td>&lt; -0.1750</td>
<td>50.0 (37.0 to 63.0)</td>
<td>76.2 (63.8 to 86.0)</td>
<td>26.2</td>
<td>0.64</td>
</tr>
<tr>
<td>T1-A</td>
<td>58 (48 to 68)</td>
<td>&lt; -0.1371</td>
<td>45.2 (32.5 to 58.3)</td>
<td>81.0 (69.1 to 89.8)</td>
<td>26.1</td>
<td>0.69</td>
</tr>
<tr>
<td>T4-A</td>
<td>59 (49 to 69)</td>
<td>&lt; -0.0921</td>
<td>54.8 (41.7 to 67.5)</td>
<td>73.0 (60.4 to 83.4)</td>
<td>27.9</td>
<td>0.69</td>
</tr>
<tr>
<td>CT-A</td>
<td>51 (41 to 61)</td>
<td>&lt; -0.6576</td>
<td>17.7 (9.2 to 29.5)</td>
<td>92.1 (82.4 to 97.4)</td>
<td>9.8</td>
<td>0.51</td>
</tr>
<tr>
<td>LT-A</td>
<td>52 (42 to 62)</td>
<td>&lt; -0.3737</td>
<td>66.1 (53.0 to 77.7)</td>
<td>46.0 (33.4 to 59.1)</td>
<td>12.2</td>
<td>0.28</td>
</tr>
<tr>
<td>LN-A</td>
<td>60 (50 to 70)</td>
<td>&lt; -0.0571</td>
<td>82.3 (70.5 to 90.8)</td>
<td>34.9 (23.3 to 48.0)</td>
<td>17.2</td>
<td>0.50</td>
</tr>
<tr>
<td>CN-A</td>
<td>57 (47 to 67)</td>
<td>&lt; -0.4083</td>
<td>40.3 (28.1 to 53.6)</td>
<td>74.6 (62.1 to 84.7)</td>
<td>14.9</td>
<td>0.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dynamic metrics-S</th>
<th>AUC, % (95% CI)</th>
<th>Cutoff values, °C</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>Y</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC-S</td>
<td>51 (41 to 61)</td>
<td>&lt; 0.3310</td>
<td>45.2 (32.5 to 58.3)</td>
<td>63.5 (50.4 to 75.3)</td>
<td>8.7</td>
<td>0.20</td>
</tr>
<tr>
<td>MOST-S</td>
<td>57 (46 to 67)</td>
<td>&gt; 0.5252</td>
<td>45.2 (32.5 to 58.3)</td>
<td>74.6 (62.1 to 84.7)</td>
<td>19.8</td>
<td>0.49</td>
</tr>
<tr>
<td>MinT-S</td>
<td>55 (44 to 65)</td>
<td>&gt; 0.3363</td>
<td>67.7 (54.7 to 79.1)</td>
<td>46.0 (33.4 to 59.1)</td>
<td>13.8</td>
<td>0.32</td>
</tr>
<tr>
<td>MaxT-S</td>
<td>64 (55 to 74)</td>
<td>&gt; 0.1571</td>
<td>54.8 (41.7 to 67.5)</td>
<td>68.3 (55.3 to 79.4)</td>
<td>23.1</td>
<td>0.53</td>
</tr>
<tr>
<td>T1-S</td>
<td>59 (48 to 69)</td>
<td>&gt; 0.1531</td>
<td>48.4 (35.5 to 61.4)</td>
<td>77.8 (65.5 to 87.3)</td>
<td>26.2</td>
<td>0.66</td>
</tr>
<tr>
<td>T4-S</td>
<td>61 (51 to 71)</td>
<td>&gt; 0.0004</td>
<td>62.9 (49.7 to 74.8)</td>
<td>58.7 (45.6 to 71.0)</td>
<td>21.6</td>
<td>0.49</td>
</tr>
<tr>
<td>CT-S</td>
<td>53 (43 to 63)</td>
<td>&gt; 0.0101</td>
<td>79.0 (66.8 to 88.3)</td>
<td>33.3 (22.0 to 46.3)</td>
<td>12.4</td>
<td>0.35</td>
</tr>
<tr>
<td>LT-S</td>
<td>52 (42 to 62)</td>
<td>&gt; 0.3985</td>
<td>59.7 (46.5 to 72.0)</td>
<td>49.2 (36.4 to 62.1)</td>
<td>8.9</td>
<td>0.20</td>
</tr>
<tr>
<td>LN-S</td>
<td>61 (51 to 71)</td>
<td>&gt; 0.3398</td>
<td>64.5 (51.3 to 76.3)</td>
<td>57.1 (44.1 to 69.5)</td>
<td>21.7</td>
<td>0.49</td>
</tr>
<tr>
<td>CN-S</td>
<td>58 (48 to 68)</td>
<td>&gt; 0.3175</td>
<td>48.4 (35.5 to 61.4)</td>
<td>68.3 (55.3 to 79.4)</td>
<td>16.6</td>
<td>0.39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dynamic metrics-GR</th>
<th>AUC, % (95% CI)</th>
<th>Cutoff values, °C</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>Y</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC-GR</td>
<td>54 (44 to 64)</td>
<td>&gt; -0.1771</td>
<td>61.3 (48.1 to 73.4)</td>
<td>52.4 (39.4 to 65.1)</td>
<td>13.7</td>
<td>0.31</td>
</tr>
<tr>
<td>MOST-GR</td>
<td>52 (42 to 63)</td>
<td>&lt; -0.2436</td>
<td>37.1 (25.2 to 50.3)</td>
<td>74.6 (62.1 to 84.7)</td>
<td>11.7</td>
<td>0.30</td>
</tr>
<tr>
<td>MinT-GR</td>
<td>55 (45 to 65)</td>
<td>&lt; -0.0322</td>
<td>83.9 (72.3 to 92.0)</td>
<td>30.2 (19.2 to 43.0)</td>
<td>14.0</td>
<td>0.45</td>
</tr>
<tr>
<td>MaxT-GR</td>
<td>53 (43 to 64)</td>
<td>&gt; -0.3078</td>
<td>85.5 (74.2 to 93.1)</td>
<td>33.3 (22.0 to 46.3)</td>
<td>18.8</td>
<td>0.60</td>
</tr>
<tr>
<td>T1-GR</td>
<td>55 (44 to 65)</td>
<td>&gt; -0.1719</td>
<td>71.0 (58.1 to 81.8)</td>
<td>46.0 (33.4 to 59.1)</td>
<td>17.0</td>
<td>0.41</td>
</tr>
<tr>
<td>T4-GR</td>
<td>50 (40 to 61)</td>
<td>&gt; -0.6860</td>
<td>90.3 (80.1 to 96.4)</td>
<td>17.5 (9.1 to 29.1)</td>
<td>7.8</td>
<td>0.37</td>
</tr>
<tr>
<td>CT-GR</td>
<td>56 (46 to 66)</td>
<td>&lt; -0.4405</td>
<td>33.9 (22.3 to 47.0)</td>
<td>79.4 (67.3 to 88.5)</td>
<td>13.2</td>
<td>0.37</td>
</tr>
<tr>
<td>LT-GR</td>
<td>51 (41 to 61)</td>
<td>&lt; -0.1985</td>
<td>58.1 (44.9 to 70.5)</td>
<td>52.4 (39.4 to 65.1)</td>
<td>10.4</td>
<td>0.23</td>
</tr>
<tr>
<td>LN-GR</td>
<td>55 (45 to 65)</td>
<td>&lt; -0.3620</td>
<td>27.4 (16.9 to 40.2)</td>
<td>87.3 (76.5 to 94.4)</td>
<td>14.7</td>
<td>0.53</td>
</tr>
<tr>
<td>CN-GR</td>
<td>56 (46 to 66)</td>
<td>&gt; -0.4496</td>
<td>85.3 (73.8 to 93.0)</td>
<td>28.6 (17.9 to 41.4)</td>
<td>13.8</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 6.3 shows a summary of AUC, sensitivity, specificity, Youden’s index, DP and the selected cutoff values for the 30 dynamic metrics. All dynamic metrics were shown to be of low accuracy, with AUC below 70% (Wians, 2009). Calculating Youden’s index and DP had confirmed the detector’s performance. The DP for all dynamic metrics ranged from 0.20 to 0.69 indicating poor
discrimination performance as they were less than 1 (Sokolova et al., 2006). Youden’s index was also found to be low for all dynamic metrics ranging 8.6 to 27.9 (Table 6.3).

**Phase 2: Evaluating the best combined temperature metrics in screening DED**

AUC for static metrics at 0 s can be increased to 71% by a combined metrics in the expression of $0.95\text{GCC} + 0.19\text{MOST} + 0.26\text{MinT} + 0.50\text{MaxT} + 0.09\text{T1} + 0.94\text{T4} + 0.04\text{CT} + 0.18\text{LT} + 0.07\text{LN} + 0.37\text{CN}$.

AUC for static metrics at 5 s can be increased to 72% by a combined metrics in the expression of $0.02\text{GCC} + 0.15\text{MOST} + 0.04\text{MinT} + 0.65\text{MaxT} + 0.27\text{T1} + 0.90\text{T4} + 0.26\text{CT} + 0.12\text{LT} + 0.11\text{LN} + 0.19\text{CN}$.

AUC for static metrics at 10 s can be increased to 73% by a combined metrics in the expression of $0.03\text{T1} + 0.98\text{T4} + 0.06\text{CT} + 0.02\text{LT} + 0.02\text{LN} + 0.05\text{CN}$.

It was shown that T4 was the main contributor to AUC for static metrics at all three time points. However, by just looking at the AUC at respective metric (without combining them), the AUC for T4 were shown to be very similar: 69% at 0 s, 72% at 5 s and 73% at 10 s (Table 6.2). It was therefore concluded that combining metrics was not able to meaningfully improve AUC for static measures.

On the other hand, AUC for dynamic metrics for asymptote can be increased to 53% by a combined metrics in the expression of $0.31\text{GCC} + 0.34\text{MOST} + 0.55\text{MinT} + 0.23\text{MaxT} + 0.28\text{T1} + 0.87\text{T4} + 0.84\text{CT} + 0.25\text{LT} + 0.02\text{LN} + 0.68\text{CN}$.

AUC for dynamic metrics for scale can be increased to 56% by a combined metrics in the expression of $0.21\text{GCC} + 0.06\text{MOST} + 0.04\text{MinT} + 0.06\text{MaxT} + 0.92\text{T4}$.

AUC for dynamic metrics for growth rate can be increased to 51% by 1.00T4. It was again shown that, T4 was the main contributor to AUC for dynamic metrics at all three attributes. Similarly, by just looking at the AUC at respective metric (without combining them), the AUC for T4 were shown to be very similar: 59% for asymptote, 61% for scale and 50% for growth rate (Table 6.3). It was again concluded that combining metrics was not able to meaningfully improve the AUC for dynamic measures.
Figure 6.4. ROC curves for T4 metrics (static). AUC are shown at the legend.

Figure 6.5. ROC curves for T4 metrics (dynamic). AUC are shown at the legend.

ROC curves on T4 metrics alone were then plotted and shown in Figure 6.4 and 6.5 with a summary of their AUC. It was clear that T4 metrics (static) were good detectors for DED as points were far above the diagonal line in ROC curve (Bland and Altman, 1994) (Fig. 6.4) as compared to T4 metrics (dynamic) where points were on / fairly above the diagonal line (Fig. 6.5) indicating poor detectors for DED (Bland and Altman, 1994).
6.5 Discussion

This is the first study to demonstrate that measuring temperature at the extreme nasal conjunctiva was able to discriminate mild to moderate dry eye from non-DED patients. The test was comparable to other well established methods in testing tear stability based on different principles, such as tear break-up time (BUT) (Lemp et al., 2011, Kim et al., 2015, Yeh et al., 2015) and non-invasive tear break-up time (NIBUT) (Yeh et al., 2015) (Table 4). A cutoff value of 34.7 °C for T4-5 temperature has given higher sensitivity as compared to wet BUT and dry BUT (Kim et al., 2015), BUT (Lemp et al., 2011, Yeh et al., 2015) and NIBUT (Yeh et al., 2015). On the other hand, a cutoff value of 34.5 °C for T4-10 temperature had lower sensitivity in general when compared with above-mentioned tests but its specificity was better than wet BUT (Kim et al., 2015), TBUT (Lemp et al., 2011, Yeh et al., 2015) and NIBUT (Yeh et al., 2015) (Table 6.4).

Table 6.4. A comparison of ocular thermography to similar tests found in the literature.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Tests</th>
<th>Cutoff values</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study</td>
<td>T4-5 temperature</td>
<td>34.7 °C</td>
<td>87.1</td>
<td>50.8</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>T4-10 temperature</td>
<td>34.5 °C</td>
<td>77.6</td>
<td>61.9</td>
<td>73</td>
</tr>
<tr>
<td>Kim et al., 2015</td>
<td>Wet BUT</td>
<td>4.48 s</td>
<td>79.0</td>
<td>54.8</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Dry BUT</td>
<td>3.50 s</td>
<td>72.6</td>
<td>69.4</td>
<td>72</td>
</tr>
<tr>
<td>Lemp et al., 2011</td>
<td>TBUT</td>
<td>&lt; 10 s</td>
<td>84.4</td>
<td>45.3</td>
<td>-</td>
</tr>
<tr>
<td>Yeh et al., 2015</td>
<td>NIBUT</td>
<td>-</td>
<td>72.0</td>
<td>52.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>TBUT</td>
<td>-</td>
<td>68.0</td>
<td>57.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Static metrics were found to have better efficacy in diagnosing DED as compared to dynamic metrics. It was sufficient to perform the test singly as combined metrics was not successful to increase its performance. The limited discrimination performance of any single method highlights the complexity of diagnosing DED. The 30 tested dynamic metrics were shown to have low efficacy in diagnosing DED (singly and in combination) and deserved no further discussion.

It has been reported that temperature at the conjunctiva was higher than that of the central corneal (Mapstone, 1968d, Alio and Padron, 1982). Although the reasons remains unclear, the temperature of the nasal conjunctiva was reported to be higher than that of the temporal conjunctiva because of the influence of greater blood flow and vascularization in the nasal conjunctiva (Kamao et al., 2011). More large vessels (eg. the dorsal nasal artery and the angular artery) are situated at the nasal side of the eye. In addition, the medial rectus muscle has two anterior ciliary arteries, whereas the lateral rectus muscle has only one artery. All these anatomical factors have caused a more vascularised nasal conjunctiva with higher blood flow (Kamao et al., 2011), and hence causing a higher evaporation rate / less stable tear film at T4. Further studies would be required to confirm these postulation. Nevertheless, the temperature changes at nasal conjunctiva may also be contributed by other factors such as allergy rather than dry eye and warranted further investigations.
This study was in agreement with past studies (Kamao et al., 2011, Su et al., 2011). i.e., measuring OST can be a good diagnostic tool for dry eye. Sensitivity and specificity of Tomey IR thermographer tested at GCC was reported to be 83% and 80% respectively using a cutoff value of 0.13 °C but reduced to 80% and 73% respectively using a cutoff value of 0.11 °C (Kamao et al., 2011). The values derived in Kamao et al. (2011)’s study were not defined clearly but believed was a decrease in dynamic temperature over 10 seconds (for cutoff value of 0.13 °C) and 5 seconds (for cutoff value of 0.11 °C) respectively. Using a custom-designed IR thermal image system, sensitivity and specificity of a combined temperature metrics (temperature difference and compactness values) were 84% and 83% respectively with unclear cutoff value (Su et al., 2011). Limited temperature metrics were included in the above mentioned studies as compared to sixty temperature metrics in the current report. Different in findings as compared to the current report could be due to different in methodology and subjects recruited. Ocular thermographers with lower resolution of 400 (H) x 240 (V) pixels and 320 (H) x 240 (V) pixels in Kamao et al. (2011)’s and Su et al. (2011)’s studies respectively may have hindered the visibility of the anatomical structures of the ocular surface and created errors in OST acquisition. Dry eye subjects recruited by both studies were much older (mean age 52.9 years for dry eye and 42.7 years for control subjects (Kamao et al., 2011) and mean age of 49 years for dry eye and 34 years for controls (Su et al., 2011) and the two groups were not age-matched. This is important because OST was reported to decrease with age at a rate of - 0.010 °C / year (Morgan et al., 1999). Differences in OST between groups may be age-related rather than to the disease itself.

Other tests evaluating DED such as tear osmolarity and tear evaporation rate measurement have been reported to have better diagnostic ability. Using a threshold value of 312 mOsms/L, tear osmolarity had sensitivity of 73% and specificity of 92% as compared to other tests (54% for corneal staining, 60% for conjunctival staining and 61% for meibomian gland grading on sensitivity; 45% for tear break-up time and 51% for Schirmer test on specificity (Lemp et al., 2011). On the other hand, values of 41 g/m(-2)/h for tear evaporation rate has found to give sensitivity of 96% and specificity of 67% (Khanal et al., 2008).

This study shows the diagnostic ability for IR ocular thermography in screening DED. All dry eye patients were mild to moderate with no inflamed meibomian glands. It was acknowledged that many disease severity criteria are confounded by complex disease subtypes and a lack of standardisation, and the selection of single criteria for assessment of disease severity is therefore fraught with difficulties (DEWS, 2007b, Schein et al., 1997, Sullivan et al., 2010).

The results of this study suggest that IR ocular thermography is a suitable test to be incorporated in the non-invasive diagnostic assessment of dry eye. It is repeatable, rapid, and easy to use and in this study, shown to give good sensitivity of specificity in diagnosing DED. Future studies on dry eye screening using ocular thermography should include temperature of the conjunctiva. T4-5 and
T4-10 metrics are simple, static measures and it was speculated that they could be used in combination with other conventional tests to further refine diagnostic discrimination for DED. Since this was a preliminary study, it was attempted to standardize every aspect of the testing protocol (e.g., temperature and humidity controlled room, 20 minutes of room acclimation) in order to reduce testing variability as much as possible. While this is a common research strategy, additional work is needed to determine if the findings hold up in a normal clinic environment. Nevertheless, the overall findings do indicate that ocular thermography may be useful for understanding dry eye in clinical practice.

6.6 References


7 Diagnostic ability of conventional dry eye tests and their correlation with ocular surface temperature

Contributions
I designed this study in collaboration with my supervisors and co-authors. I was solely responsible for participant recruitment and data collection. I also analysed the data with useful guidance from my supervisors. I wrote the manuscript with helpful comments from my supervisors and co-authors.

Publications

Conference presentations
Abstract of this work has been accepted for poster presentation at a scientific session of American Academy of Optometry (AAO) 9-12 September 2016, Anaheim Convention Center, USA.

Acknowledgements
This study was funded by Singapore ToteBoard Organisation no. LS/CLS/TM/2009/007. The authors thank Dr. Cai Zhi Qiang from School of Electronic and Electrical Engineering in writing the OST analysis V2 program using MatLab Simulink 7.11.0 (R2010b) and Mr Peter Mylon from the University of Manchester for his valuable statistical advice.
7.1 Abstract

7.1.1 Purpose
To study the diagnostic ability of conventional dry eye tests and their correlation with ocular surface temperature (OST) and derive the best combined objective tests for dry eye.

7.1.2 Methods
This was a single visit study included a few conventional dry eye tests on 62 dry eye and 82 control subjects: symptom evaluation, fluorescein break-up time (FBUT), corneal epithelial staining (CES), non-invasive break-up time (NIBUT) and tear meniscus height (TMH). OST was recorded using NEC thermo tracer TH 9260 and six temperature metrics of the extreme nasal conjunctiva was studied including the temperature 10 seconds after eye opening (T4-10). Diagnostic ability was assessed by calculating sensitivity and specificity and area under the receiver operating characteristics curve (AUC).

7.1.3 Results
No correlation (Pearson's coefficient, -0.203 to 0.209; p > 0.05) was found between Mscore, Scount, FBUT and CES with any of the temperature metrics. However, CES correlated significantly with TMH (r = 0.276; p = 0.030) and inversely correlated significantly with FBUT (r = - 0.258; p = 0.043). Values of Mscore at 8 were found to give sensitivity of 87.1% (95% CI: 76.2 to 94.3%) and specificity of 92.7% (84.8 to 97.3%). Values of Scount at 1 were found to give sensitivity of 93.6% (84.3 to 98.2%) and specificity of 65.9% (54.6 to 76.0%). Values of FBUT at 2 s were found to give sensitivity of 58.1% (44.9 to 70.5%) and specificity of 87.8% (78.7 to 94.0%). Values of CES at grade 2 were found to give sensitivity of 71% (58.1 to 81.8%) and specificity of 59.8% (48.3 to 70.4%). Combining CES with T4-10 (series) had increased the AUC to 78% with sensitivity and specificity of 92.3% and 42.7% respectively.

7.1.4 Conclusions
This work validated the ability of Mscore, Scount, FBUT and CES in diagnosing dry eye and further confirmed the discordance between its signs and symptoms. Combining CES with T4-10 (series) can be future objective tests for dry eye.

Keywords. Correlation, dry eye diagnosis, conventional dry eye tests, ocular surface temperature, temperature metrics, sensitivity, specificity.
7.2 Introduction

While there are many tests available for dry eye disease (DED), it was well understood that eye care practitioners generally rely on a battery of tests for its diagnosis (Nichols et al., 2000). Surveys on DED diagnostic testing indicate that there is no one single test which dominates (Korb, 2000) although many practitioners rank symptom reporting as their preferred test (Korb, 2000, Downie et al., 2013). Conventional clinical tests for DED diagnosis include fluorescein tear break-up time, corneal fluorescein staining, and meibomian gland evaluation (Downie et al., 2013) and it is generally agreed that there is a lack of consistency between such measures (Korb, 2000, Downie et al., 2013). The situation is further complicated in that DED is multifactorial, determining the cause of dry eye with minimal clinical signs is difficult, and there is a lack of correlation between symptoms and objective tests (Nelson et al., 2000, Begley et al., 2003). It is unlikely that a single test can provide a complete assessment of DED (Tomlinson et al., 2011) and in recent international workshops, multiple tests have been advocated for DED diagnosis therapy evaluation (DEWS 2007a, Tomlinson et al., 2011).

Alterations to tear film stability is generally accepted as a key feature of DED (DEWS 2007a). Ocular surface temperature (OST) measurement with infrared (IR) ocular thermography is indicative of the tear film and its stability (Craig et al., 2000, Labbe et al., 2007). Ocular thermography has been used to study DED (Morgan et al., 1993, 1995, 1996, Fujishima et al., 1996, Mori et al., 1997, Craig et al., 2000, Zelichowska et al., 2005, Singh and Bhinder, 2005, Kamao et al., 2011, Su et al., 2011) with three studies reporting on its diagnostic ability (Zelichowska et al., 2005, Kamao et al., 2011, Su et al., 2011) with inconsistent results. The application of IR ocular thermography in screening mild to moderate DED was considered. A region of the ocular surface showing the greatest diagnostic potential was the extreme nasal conjunctiva, especially when evaluated ten seconds after eye opening (Tan et al., 2016a). In common with previous reports (Tan et al., 2016a), this region was referred as ‘T4’ in this manuscript. The correlation between T4 with dry eye symptoms and conventional objective clinical dry eye tests was not previously considered; furthermore the diagnostic performance of combining this form of thermographic measure with conventional approaches has not previously been studied. As such, the current study was designed to address both of these issues.

7.3 Methods

7.3.1 Subjects

The research protocol was approved by the Singapore National Health Group (NHG) Domain-Specific Review Board (DSRB) and the Singapore Polytechnic ethics review committee and the work adhered to the tenets of the Declaration of Helsinki. A total of 62 dry eye (mean ± standard deviation age 48 ± 10 years; 14 males and 48 females) and 82 control subjects (aged 44 ± 7 years; 35 males and 47 females) were recruited. Informed consent was obtained from each subject at
study enrolment. The inclusion criteria for the dry eye subjects were as described previously (Tan et al., 2016b): use of tear replacement therapy and had either a fluorescein tear break-up time of 10 seconds or less (Golding and Brennan, 1993), or a Schirmer I test result of less than 10 mm in 5 min (Morgan et al., 1995) along with presence of corneal or conjunctiva staining. All dry eye patients were screened and diagnosed by an ophthalmologist at Khoo Tech Puat Hospital eye clinic prior to starting the study. Classification of mild or moderate and severe patients was based on a composite disease severity index, derived from the Dry Eye Workshop severity scale (DEWS 2007b). Control subjects were those not using tear replacement therapy or any topical medication and without signs or symptoms of dry eye. All subjects were required to have not worn contact lenses for at least two years prior to enrolment. Subjects were excluded from the control group if they had Schirmer I test result of less than 10 mm in 5 min or fluorescein tear break-up time of 10 seconds or less. Subjects with any anterior ocular anomalies (e.g. current ocular infection, allergy or ptosis), those undergone surgery or taking any medication that could affect the tear film or who were currently pregnant or breastfeeding were also excluded (Tan et al., 2016b).

### 7.3.2 Procedures

In this single visit study, a number of practitioner-preferred (Korb, 2000) conventional clinical tests were performed on both eyes: symptom evaluation using McMonnies dry eye questionnaire (Mscore) and symptoms count (Scount), Fluorescein break-up time (FBUT), fluorescein corneal epithelial staining (CES), non-invasive break-up time (NIBUT) and the lower tear meniscus height (TMH).

McMonnies dry eye questionnaire (DEQ) was used in this study as it is long-standing, widely used and reported to be efficient to screen DED (Gothwal et al., 2010). Indeed, it is regarded as the “gold standard” questionnaire for dry eye and is statistically reliable and repeatable (Erickson et al., 2002). Subjects were interviewed to complete the McMonnies DEQ consisting of 12 questions (McMonnies and Ho, 1987) and the total score was calculated using the DEWS dry eye diagnostic template (DEWS, 2007a). On the other hand, Scount was the number of symptoms based on McMonnie’s DEQ Q2 (soreness, scratchiness, dryness, grittiness, burning) (McMonnies and Ho, 1986, McMonnies and Ho, 1987) with each symptom afforded a score of one point, to a maximum of five.

A Topcon DC-1 slit lamp biomicroscope was used to assess the anterior ocular health, FBUT, CES and TMH. A drop of fluorescein sodium HCL was instilled on the subject’s eye and the cornea and tear film were assessed using cobalt blue light, viewed through a yellow barrier filter (Wratten #12) for FBUT and CES. FBUT was the time taken for the first dark spot to appear after a complete blink as suggested by previous workers (Norn, 1969, Lemp and Holly, 1970, Lemp and Hamill, 1973). FBUT was recorded on both eyes and the average of the first three readings were used. CES was recorded and graded according to Lemp’s scale. Lemp’s scale was opted rather than the van Bijsterveld system (VanBijsterveld, 1969) or the Oxford system (Bron et al., 2003) because it is
widely used and has been adopted as a standard by the National Eye Institute / Industry Workshop (Lemp, 1995). According to Lemp’s scale, the cornea is divided into 5 regions, with each being graded from 0 to 3. The scores for the 5 regions were summed up and recorded. In this study, fluorescein was used to assess corneal staining as it has been reported as being highly sensitive for dry eye diagnosis (Whitcher, 1987).

NIBUT was measured using a computerized High-Speed videokeratoscope (Medmont E300) which uses 32 rings and over 15,000 measurement points over a wide area of the human cornea, with Medmont studio version 4.12.0 (Medmont International Pty Ltd. Australia). The method reported by Iskander and Collins (2005) who analysed tear film stability in the inter-blink interval, and measured tear film break-up time was adopted. While fixating at the center of a series of red placido rings, the subjects blink normally, closed for 3 s, open widely and hold blink for 10 s. NIBUT was recorded as the time required for the first appearance of distorted HSV mires. A number of repeated measures were recorded for both eyes and the average of the best three readings was used.

TMH was photographed using IMAGEnet software (Topcon medical systems, Inc., Oakland, NJ) and measured as reported by Kwong and Cho (2001). The slit lamp eye piece and illumination lamp were positioned perpendicular to the lower tear meniscus. Subjects were asked to look straight ahead while a 1 mm conical beam at 25x magnification and medium illumination was placed at the center of the lower tear meniscus. TMH readings were measured using calibrated software (Adobe Photoshop CS2).

OST was recorded using NEC thermo tracer TH 9260 using a previously-described method which has been shown to be repeatable when assessing healthy and dry eyes (Tan et al., 2016b). Six temperature metrics were used which related to the temperature of the extreme nasal conjunctiva (T4). These were the temperature immediately on eye opening and five and ten seconds after opening (T4-0, T4-5, T4-10) and then three metrics related to exponential curve fit of the change in temperature of this location after eye opening (T4-A, T4-S and T4-GR).

These latter variables represent the output variables when a one phase exponential curve is fitted to T4 temperature vs. time using JMP version 12.1.0 (http://www.jmp.com; SAS Institute Inc., USA) according to the model:

\[ \text{Temperature} = a + b \times \text{Exp} (c \times \text{time}) \]

where ‘a’ represents the asymptote of the best fit curve (‘T4-A’), ‘b’ is the ‘scale’ (‘T4-S’) and ‘c’ is the ‘growth rate’ (‘T4-GR’).

### 7.3.3 Data analysis

Data on 62 dry eye and 82 control subjects were tabulated and analysed. Only data obtained from right eye were used in the analysis to prevent difficulties arising when non-independent data were collected from both eyes (Ray and O’Day, 1985).
Correlation between T4 metrics and signs and symptoms for dry eye

Unpaired t-tests (two-tailed) were first performed to explore differences between dry eye and control subjects for each of the conventional clinical test. Multivariate analysis followed by Pearson correlation test were then performed on dry eye subjects using JMP version 12.1.0 (http://www.jmp.com; SAS Institute Inc., USA) to explore correlations between T4 metrics with dry eye symptoms and conventional objective clinical tests. All the above analysis were done at 95% confidence. Multivariate statistical methods enabled analysis of complex datasets where several outcomes variables are measured and known to be related and to have an effect on each other and is useful to explain complex clinical situations in simpler ways (Tomlinson et al., 2013). In this sort of analysis, large datasets are recommended and sample sizes are often said to be appropriate when the number of subjects is 5 or even 10 times the number of outcome measures (Tomlinson et al., 2013). As there were 12 main outcomes measures in the current study, a minimum of 60 subjects should be available.

Diagnostic ability of conventional clinical tests and combining with T4 metrics

Diagnostic ability of the conventional clinical tests were evaluated in terms of their sensitivity, specificity and area under the receiver operating characteristics curves (AUC) (Bland and Altman, 1994a). Cutoff values, discrimination power (DP) (Sokolova et al., 2006) and Youden's index (Y) (Youden WJ, 1950) for all tests were also studied as described in the previous report (Tan et al., 2016a). The clinical tests with best performance were combined with T4 metrics to ascertain if AUC of the combined tests could be improved. Deriving the best combination of conventional and thermographic tests was undertaken by using the Solver function of Microsoft EXCEL (Microsoft EXCEL 2013, USA). The analysis was developed for each dataset in order to reduce the dimensionality of the variables down to one or two factors combining these variables and determine the best detector(s).

7.4 Results

Mean and standard deviations of the values obtained on the six conventional clinical tests for DED in dry eye and control subjects are shown in Table 7.1. Significant differences were found between the two groups on four tests: FBUT, CES, Mscore and Scount (unpaired t-test, p < 0.0001) at 95% CI. Dry eye subjects had significantly shorter FBUT but greater CES, Mscore and Scount as compared to control subjects.
Table 7.1. Results of conventional clinical tests in dry eye and control subjects.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Dry eye</th>
<th>Control</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBUT (s)</td>
<td>2.6 ± 2.2</td>
<td>4.5 ± 2.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NIBUT (s)</td>
<td>2.6 ± 3.8</td>
<td>2.5 ± 3.5</td>
<td>0.794</td>
</tr>
<tr>
<td>TMH (mm)</td>
<td>0.18 ± 0.08</td>
<td>0.19 ± 0.09</td>
<td>0.566</td>
</tr>
<tr>
<td>CES (grade)</td>
<td>2.7 ± 3.0</td>
<td>1.0 ± 1.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mscore</td>
<td>10.2 ± 3.4</td>
<td>2.4 ± 2.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Scount</td>
<td>2.0 ± 1.1</td>
<td>0.5 ± 0.7</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Data are the mean ± SD.

Correlation between T4 metrics and signs and symptoms for dry eye

No correlation was found between FBUT, CES, Mscore and Scount with any of the T4 metrics in dry eye (Pearson's coefficient, - 0.203 to 0.209; p > 0.05) (Table 7.2) or control subjects (Pearson's coefficient, - 0.223 to 0.194; p > 0.05) (Table 7.3).

Table 7.2. Correlations between T4 metrics and conventional clinical tests for dry eye subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>by Variable</th>
<th>r values</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBUT</td>
<td>T4-0</td>
<td>0.006</td>
<td>-0.245</td>
<td>0.255</td>
<td>0.966</td>
</tr>
<tr>
<td></td>
<td>T4-5</td>
<td>0.027</td>
<td>-0.225</td>
<td>0.275</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>T4-10</td>
<td>-0.023</td>
<td>-0.279</td>
<td>0.237</td>
<td>0.865</td>
</tr>
<tr>
<td></td>
<td>T4-A</td>
<td>-0.161</td>
<td>-0.395</td>
<td>0.092</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>T4-S</td>
<td>0.207</td>
<td>-0.045</td>
<td>0.434</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td>T4-GR</td>
<td>0.047</td>
<td>-0.205</td>
<td>0.294</td>
<td>0.715</td>
</tr>
<tr>
<td>CES</td>
<td>T4-0</td>
<td>0.196</td>
<td>-0.057</td>
<td>0.425</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>T4-5</td>
<td>0.209</td>
<td>-0.043</td>
<td>0.436</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>T4-10</td>
<td>0.172</td>
<td>-0.090</td>
<td>0.412</td>
<td>0.197</td>
</tr>
<tr>
<td></td>
<td>T4-A</td>
<td>0.079</td>
<td>-0.174</td>
<td>0.323</td>
<td>0.540</td>
</tr>
<tr>
<td></td>
<td>T4-S</td>
<td>-0.137</td>
<td>-0.374</td>
<td>0.117</td>
<td>0.289</td>
</tr>
<tr>
<td></td>
<td>T4-GR</td>
<td>0.052</td>
<td>-0.200</td>
<td>0.298</td>
<td>0.668</td>
</tr>
<tr>
<td>Mscore</td>
<td>T4-0</td>
<td>0.097</td>
<td>-0.157</td>
<td>0.338</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>T4-5</td>
<td>0.172</td>
<td>-0.081</td>
<td>0.404</td>
<td>0.182</td>
</tr>
<tr>
<td></td>
<td>T4-10</td>
<td>0.188</td>
<td>-0.074</td>
<td>0.426</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td>T4-A</td>
<td>0.038</td>
<td>-0.214</td>
<td>0.285</td>
<td>0.769</td>
</tr>
<tr>
<td></td>
<td>T4-S</td>
<td>-0.153</td>
<td>-0.388</td>
<td>0.100</td>
<td>0.234</td>
</tr>
<tr>
<td></td>
<td>T4-GR</td>
<td>0.199</td>
<td>-0.053</td>
<td>0.428</td>
<td>0.121</td>
</tr>
<tr>
<td>Scount</td>
<td>T4-0</td>
<td>0.004</td>
<td>-0.246</td>
<td>0.253</td>
<td>0.976</td>
</tr>
<tr>
<td></td>
<td>T4-5</td>
<td>0.060</td>
<td>-0.193</td>
<td>0.305</td>
<td>0.645</td>
</tr>
<tr>
<td></td>
<td>T4-10</td>
<td>0.088</td>
<td>-0.174</td>
<td>0.339</td>
<td>0.511</td>
</tr>
<tr>
<td></td>
<td>T4-A</td>
<td>0.162</td>
<td>-0.092</td>
<td>0.395</td>
<td>0.209</td>
</tr>
<tr>
<td></td>
<td>T4-S</td>
<td>-0.203</td>
<td>-0.431</td>
<td>0.049</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>T4-GR</td>
<td>0.095</td>
<td>-0.158</td>
<td>0.337</td>
<td>0.462</td>
</tr>
</tbody>
</table>
Table 7.3. Correlations between T4 metrics and conventional clinical tests for control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>by Variable</th>
<th>r values</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBUT</td>
<td>T4-0</td>
<td>0.142</td>
<td>-0.078</td>
<td>0.348</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>T4-5</td>
<td>0.141</td>
<td>-0.078</td>
<td>0.348</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td>T4-10</td>
<td>0.155</td>
<td>-0.064</td>
<td>0.360</td>
<td>0.164</td>
</tr>
<tr>
<td></td>
<td>T4-A</td>
<td>-0.013</td>
<td>-0.229</td>
<td>0.205</td>
<td>0.909</td>
</tr>
<tr>
<td></td>
<td>T4-S</td>
<td>0.006</td>
<td>-0.211</td>
<td>0.223</td>
<td>0.957</td>
</tr>
<tr>
<td></td>
<td>T4-GR</td>
<td>-0.020</td>
<td>-0.236</td>
<td>0.198</td>
<td>0.859</td>
</tr>
<tr>
<td>CES</td>
<td>T4-0</td>
<td>-0.223</td>
<td>-0.420</td>
<td>-0.006</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>T4-5</td>
<td>-0.135</td>
<td>-0.342</td>
<td>0.084</td>
<td>0.226</td>
</tr>
<tr>
<td></td>
<td>T4-10</td>
<td>-0.133</td>
<td>-0.341</td>
<td>0.086</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>T4-A</td>
<td>-0.177</td>
<td>-0.379</td>
<td>0.042</td>
<td>0.112</td>
</tr>
<tr>
<td></td>
<td>T4-S</td>
<td>0.194</td>
<td>-0.024</td>
<td>0.394</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>T4-GR</td>
<td>0.101</td>
<td>-0.119</td>
<td>0.311</td>
<td>0.368</td>
</tr>
<tr>
<td>Mscore</td>
<td>T4-0</td>
<td>-0.073</td>
<td>-0.285</td>
<td>0.147</td>
<td>0.517</td>
</tr>
<tr>
<td></td>
<td>T4-5</td>
<td>-0.042</td>
<td>-0.257</td>
<td>0.177</td>
<td>0.708</td>
</tr>
<tr>
<td></td>
<td>T4-10</td>
<td>-0.041</td>
<td>-0.256</td>
<td>0.177</td>
<td>0.712</td>
</tr>
<tr>
<td></td>
<td>T4-A</td>
<td>-0.004</td>
<td>-0.221</td>
<td>0.213</td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>T4-S</td>
<td>-0.022</td>
<td>-0.237</td>
<td>0.196</td>
<td>0.848</td>
</tr>
<tr>
<td></td>
<td>T4-GR</td>
<td>0.066</td>
<td>-0.153</td>
<td>0.279</td>
<td>0.555</td>
</tr>
<tr>
<td>Scount</td>
<td>T4-0</td>
<td>0.127</td>
<td>-0.092</td>
<td>0.335</td>
<td>0.254</td>
</tr>
<tr>
<td></td>
<td>T4-5</td>
<td>0.167</td>
<td>-0.052</td>
<td>0.370</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>T4-10</td>
<td>0.152</td>
<td>-0.067</td>
<td>0.357</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>T4-A</td>
<td>-0.002</td>
<td>-0.219</td>
<td>0.215</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>T4-S</td>
<td>0.001</td>
<td>-0.216</td>
<td>0.218</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>T4-GR</td>
<td>-0.003</td>
<td>-0.220</td>
<td>0.214</td>
<td>0.981</td>
</tr>
</tbody>
</table>

Within the dry eye subjects, there were some correlations between the six conventional clinical tests. Results shown that CES correlated significantly with TMH (Pearson's coefficient, $r = 0.276; p = 0.030$) and inversely correlated significantly with FBUT (Pearson's coefficient, $r = -0.258; p = 0.043$) (Table 7.4). Symptoms (Mscore and Scount) for dry eye did not correlate with any of the objective tests studied. FBUT was not correlated with NIBUT although both measured tear film stability (Pearson's coefficient, $r = -0.085; p = 0.547$) (Table 7.4).
Table 7.4. Correlations among conventional clinical tests for dry eye subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>by Variable</th>
<th>$r$ values</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>$p$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIBUT</td>
<td>FBUT</td>
<td>-0.085</td>
<td>-0.347</td>
<td>0.190</td>
<td>0.547</td>
</tr>
<tr>
<td>TMH</td>
<td>FBUT</td>
<td>-0.059</td>
<td>-0.304</td>
<td>0.194</td>
<td>0.649</td>
</tr>
<tr>
<td></td>
<td>NIBUT</td>
<td>0.084</td>
<td>-0.191</td>
<td>0.346</td>
<td>0.551</td>
</tr>
<tr>
<td>CES</td>
<td>FBUT</td>
<td>-0.258</td>
<td>-0.477</td>
<td>-0.008</td>
<td>0.043*</td>
</tr>
<tr>
<td></td>
<td>NIBUT</td>
<td>-0.023</td>
<td>-0.291</td>
<td>0.249</td>
<td>0.870</td>
</tr>
<tr>
<td></td>
<td>TMH</td>
<td>0.276</td>
<td>0.028</td>
<td>0.492</td>
<td>0.030*</td>
</tr>
<tr>
<td>MScore</td>
<td>FBUT</td>
<td>-0.121</td>
<td>-0.360</td>
<td>0.133</td>
<td>0.350</td>
</tr>
<tr>
<td></td>
<td>NIBUT</td>
<td>0.247</td>
<td>-0.025</td>
<td>0.485</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>TMH</td>
<td>0.152</td>
<td>-0.101</td>
<td>0.387</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>CES</td>
<td>0.128</td>
<td>-0.126</td>
<td>0.366</td>
<td>0.322</td>
</tr>
<tr>
<td>Scount</td>
<td>FBUT</td>
<td>-0.035</td>
<td>-0.282</td>
<td>0.217</td>
<td>0.790</td>
</tr>
<tr>
<td></td>
<td>NIBUT</td>
<td>0.112</td>
<td>-0.163</td>
<td>0.371</td>
<td>0.426</td>
</tr>
<tr>
<td></td>
<td>TMH</td>
<td>0.016</td>
<td>-0.235</td>
<td>0.265</td>
<td>0.901</td>
</tr>
<tr>
<td></td>
<td>CES</td>
<td>-0.010</td>
<td>-0.259</td>
<td>0.240</td>
<td>0.937</td>
</tr>
</tbody>
</table>

*p<0.05

Diagnostic ability of conventional clinical tests and combining with T4 metrics

Figure 7.1. ROC curves for conventional clinical tests. AUC are shown at the legend.

Figure 7.1 shows the ROC curves for the six conventional clinical tests. From each of the ROC curves, AUC was extracted using trapezoidal numerical integration. Mscore provided the greatest AUC at 97% suggesting good diagnostic accuracy with AUC above 90% (Wians, 2009). Scount and FBUT had AUC of 86% and 76%, respectively, suggestive of moderate accuracy with AUC lies between 70 to 90%. The rest of the tests (CES, NIBUT and TMH) had AUC below 70 indicating low accuracy (Wians, 2009). Table 7.5 shows a summary of AUC, sensitivity and specificity in
descending order of test performances based on Youden’s index and discrimination power.

**Table 7.5.** Test effectiveness of the conventional clinical tests. AUC, sensitivity, specificity, Youden’s index (Ƴ), discrimination power (DP) and the selected cutoff values are shown.

<table>
<thead>
<tr>
<th>Conventional tests</th>
<th>AUC, % (95% CI)</th>
<th>Cutoff values</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>Ƴ</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mscore</td>
<td>97 (94 to 99)</td>
<td>&gt; 7</td>
<td>87.1 (76.2 to 94.3)</td>
<td>92.7 (84.8 to 97.3)</td>
<td>79.8</td>
<td>2.45</td>
</tr>
<tr>
<td>Scount</td>
<td>87 (81 to 93)</td>
<td>&gt; 0.5</td>
<td>93.6 (84.3 to 98.2)</td>
<td>65.9 (54.6 to 76.0)</td>
<td>59.4</td>
<td>1.84</td>
</tr>
<tr>
<td>FBUT (s)</td>
<td>76 (67 to 84)</td>
<td>&lt; 2.5</td>
<td>58.1 (44.9 to 70.5)</td>
<td>87.8 (78.7 to 94.0)</td>
<td>45.9</td>
<td>1.27</td>
</tr>
<tr>
<td>CES (grade)</td>
<td>68 (60 to 77)</td>
<td>&gt; 1</td>
<td>71.0 (58.1 to 81.8)</td>
<td>59.8 (48.3 to 70.4)</td>
<td>30.7</td>
<td>0.71</td>
</tr>
<tr>
<td>TMH (mm)</td>
<td>51 (41 to 61)</td>
<td>&lt; 0.25</td>
<td>85.5 (74.2 to 93.1)</td>
<td>23.2 (14.6 to 33.8)</td>
<td>8.7</td>
<td>0.32</td>
</tr>
<tr>
<td>NIBUT (s)</td>
<td>51 (41 to 62)</td>
<td>&lt; 0.4</td>
<td>54.7 (40.5 to 68.4)</td>
<td>52.4 (41.1 to 63.6)</td>
<td>7.2</td>
<td>0.16</td>
</tr>
</tbody>
</table>

For T4 temperature measured after 10 seconds of eye opening (T4-10), AUC was 73%, similar to FBUT and CES (Fig. 7.2). Table 7.6 shows a summary of AUC, sensitivity, specificity and test performances of the tested objective tests, when applied singly and in combination. Combining T4-10 with CES increased AUC to 78% in the expression of 0.30 T4-10 + 0.78 CES, with sensitivity and specificity of 92.3% and 42.7% respectively. Combining T4-10 with CES and FBUT increased AUC slightly to 79% in the expression of 0.30 T4-10 + 0.65 CES + 0.01 FBUT with sensitivity and specificity of 92.3% and 45.1% respectively. ROC curves of the combined tests are shown in Figure 7.2. FBUT adds very little to the AUC overall, T4-10 and CES was a good combination.

![Figure 7.2](image_url)  
**Figure 7.2.** ROC curves for single vs combined objective tests. AUC are shown at the legend.

**Table 7.6.** Test effectiveness of single vs combined objective tests in the diagnosis of dry eye. AUC, sensitivity, specificity, Youden’s index (Ƴ), discrimination power (DP) and the selected cutoff values are shown.
Discussion

For the conventional methods, Mscore of 8 and above, Scount of 1 and above, FBUT of 2 s or less and CES of 2 or more were able to differentiate DED subjects from controls. NIBUT and TMH, on the other hand, were not able to do so. All dry eye patients were mild to moderate with no inflamed meibomian glands. It was recognised that many disease severity criteria are confounded by complex disease subtypes and a lack of standardisation, and the selection of single criteria for assessment of disease severity is therefore fraught with difficulties (Schein et al., 1997, DEWS, 2007a, Sullivan et al., 2010).

### 7.5 Correlation between T4 metrics and signs and symptoms for dry eye

Although it was previously demonstrated that the T4 metrics have some utility in differentiating dry eyes vs. controls, the present study was in agreement with Fujishima et al. (1996) i.e., no correlation was found between OST with individual dry eye signs or symptoms.

The findings of this study were in accordance to Kamao et al. (2011)’s study who found no correlation between FBUT with conjunctival temperature. Changes in OST can be contributed by tear film instability (Craig et al., 2000, Kamao et al., 2011) and rapid tear evaporation (Mathers, 2004). Others have reported contrary findings. According to Kamao et al (2011) and Versura et al (2015), corneal temperature was found to be low in dry eye patients and decrease in temperature correlated significantly with tear break-up time, subject’s age, subjective discomfort symptoms and enhanced evaporation in evaporative dry eye (EDE) (Versura et al., 2015). Although the dry eye cohort in this study had significantly shorter FBUT, the differences in results could be due to (1) the focus on the conjunctiva in this work, not the cornea and (2) subjects were mostly mild to moderate dry eyes and not solely consist of EDE subtypes with enhanced evaporation as reported by Versura et al. (2015).

It is noteworthy that findings on correlation between OST and dry eye tests are inconsistent and contradictory. Su et al. (2014) reported a strong correlation between areas of tear film break-up with areas of lower temperature and suggested relationship between tear film break-up and evaporation in subjects with normal tear film. However, when the study was repeated on dry eye patients, no such relationship was observed (Su et al., 2015). In another study on normal subjects,
rates of ocular surface cooling was found to be positively correlated to fluorescein tear thinning and break-up (Li et al., 2015). Simultaneous imaging of OST and fluorescein adopted in these studies (Su et al., 2014, 2015, Li et al., 2015) indicated some improvements in methodology to study OST and tear film behaviour at the same time. Pattmöller et al. (2014), on the other hand, reported no correlation between corneal temperatures with other ocular parameters such as corneal thickness, endothelial cell density and anterior chamber depth in normal subjects.

This study confirmed the discordance between signs and symptoms for DED (Nelson et al., 2000, Kallarackal et al., 2002, Begley et al., 2003, Nichols et al., 2004, Moore et al., 2009, Cardona et al., 2010, Lemp et al., 2011a, Sullivan et al., 2014). Indeed, it was reported that up to 40% of patients had symptom and clinical sign discordance (Lemp et al. 2011a). A more recent study has shown that dry eye symptoms aligned more closely to non-ocular pain, depression and post-traumatic stress disorder than tear film parameters (Galor et al., 2015).

Studies on correlation among conventional clinical tests for dry eye has been undertaken for many years (Golding and Brennan, 1993, Cho and Douthwaite, 1995, Golding et al., 1997, Schein et al., 1997, Nelson et al., 2000, Kallarackal et al., 2002, Begley et al., 2003, Nichols et al., 2003, Nichols et al., 2004, Wang et al., 2008, Moore et al., 2009, Cardona et al., 2010, Lemp et al., 2011a, Cuevas et al., 2012, Sullivan et al., 2014, Tukenmez-Dikmen, 2016, Yeh et al., 2015) with inconsistent results. Such findings confirmed the complexity of DED and how the disease is multifactorial, depending on the dry eye subtypes, symptom questionnaire used and population studied (DEWS, 2007c). Additionally, there are age- and gender-related, cultural, and ethnic influences on symptoms (Schaumberg et al., 2003, Schaumberg et al., 2009, Tran et al., 2013). CES was correlated with TMH and inversely correlated with FBUT which is in agreement with (Tung et al., 2014) and Nichols et al. (2003), respectively. However, this findings could be due to possible type I errors when doing data analysis on multiple tests.

**Diagnostic ability of conventional clinical tests and combining with T4 metrics**

In this report, AUC was used as an indicator for test accuracy (Wiens, 2009) whilst Youden’s index and DP were used as indicators for test performance (Alonso-Caneiro et al., 2011). Sensitivity is the proportion of actual positives (i.e. dry eye subjects) that are correctly identified, while specificity is the proportion of actual negatives (i.e. control subjects) that are correctly identified (Bland and Altman, 1994b). Youden’s index evaluates the algorithm’s ability to avoid failure and follows the expression:

\[ Y = \text{sensitivity} + \text{specificity} - 100. \]

Its value ranges from 0 to 100 in percentage, and has a zero value when a diagnostic test gives the same proportion of positive results for groups with and without the disease, i.e the test is useless. Cutoff value of a test was determined as the criterion that maximized the Youden index: max (sensitivity + specificity - 100), where c ranges over all possible criterion values (Youden, 1950). It is a value that optimizes the test differentiating ability when equal weight is given to sensitivity
and specificity (Youden, 1950; Faraggi, 2000; Reiser, 2000). DP is a measurement that summarizes sensitivity and specificity of the technique:

$$DP = \sqrt{3/\pi} \log (X + \log Y),$$

where $X = \text{sensitivity} / (100 - \text{sensitivity})$ and $Y = \text{specificity} / (100 - \text{specificity})$. Values of $DP < 1$ indicate poor discrimination performance, $DP < 2$ indicates limited performance, $DP < 3$ considered to be a fair discrimination, while values above 3 are classified as good.

**Symptom evaluation**

The results were in agreement with previous studies that McMonnies DEQ has good test performance with sensitivity varying between 87% and 98% and specificity between 87% and 97% (Golding and Brennan, 1993, McMonnies and Ho, 1987, McMonnies et al., 1998). The variations in estimates of sensitivity reported in the literature could be due to differences in experimental population, the criteria used for dry eye classification and different scoring methods as well as variation in cutoff values ranging from 8 to 19 (McMonnies and Ho, 1987, McMonnies et al., 1998, Gothwal et al., 2010). Of course, high sensitivity values are expected given the importance of symptom assessment in the diagnosis of DED. Scount can be a supplementary test as it was part of the McMonnies DEQ; results found that patients presented with one symptom out of the five stated in McMonnie's DEQ Q2 (soreness, scratchiness, dryness, grittiness, burning) (McMonnies, 1986, 1987) can be suspected of having dry eye.

**FBUT and CES**

Findings on FBUT and CES were in agreement to Downie et al. (2013)’s report suggesting that FBUT and CES can be treated as key clinical objective tests with good sensitivity and specificity. Cutoff value for CES in this study was similar to that reported in the literature (Lemp et al., 2011b), with higher sensitivity but lower specificity. Lemp et al. (2011b) reported that only 1 in 4 severe dry eye subjects showed little or no evidence of staining. DED can present without keratitis (Yokoi et al., 2011) and therefore it is possible to have very little CES present in dry eye patients. Findings for FBUT vary across the literature due to different cutoff values; this study has derived reasonable good sensitivity and specificity with cutoff < 2.5 s.

**NIBUT and TMH**

FBUT and NIBUT were poorly correlated, consistent with Cho and Douthwaite (1995)’s study. Variation in findings for NIBUT in the literature can generally be explained by the range of different techniques employed (Mengher et al., 1985, Cho, 1993, Nichols et al., 2002, Kojima et al., 2004, Yokoi and Komuro, 2004). NIBUT measured using high-speed videokeratoscope in the current study seem to give lower sensitivity and specificity than other work has reported. Differences in subject gender and ethnicity may also cause variation in results (Yeh et al., 2015). Subjects were mainly Asian, so the findings may be different from those studied primarily on Caucasian eyes. Reports on TMH have also been contradictory, again due to differences in the measurement techniques, ranging from photographing an optic section of the inferior tear meniscus (Mainstone et
al., 1996) to using anterior segment optical coherence tomography (Gumus and Pflugfelder, 2013). Findings in this study was similar to Kwong and Cho (2001)’s report using the same technique.

Combining thermography findings with conventional measures
Good diagnostic power when CES combined with T4-10 thermographic measure was demonstrated as two clinical measures, in conjunction with routine symptomatology. In particular it is of note that thermography provided similar outcomes to FBUT, with both tests indicative of the stability of the tear film.

FBUT assessment, of course, requires a high degree of clinical skill and the use of fluorescein drops or strips. On the other hand, thermography provides a near-immediate non-invasive assessment. As thermography has a similar test accuracy to FBUT and CES, symptomatology with thermography can be future dry eye diagnostic tests for non-clinician.

There are various problems when comparing findings across the dry eye literature. In particular, selection and spectrum bias (Tomlinson et al., 2013) is a concern. All the dry eye patients in the current study were of mild to moderate severity which might point towards some selection bias in this cohort (Gilbard and Farris, 1979, Farris et al., 1983). However, any bias was minimised by carefully adopting the same recruitment and assessment techniques for all subjects, as advocated by Tomlinson et al. (2013).

In common with previous work, multiple tests for dry eye disease are more useful than single tests (Khanal et al., 2008, Tomlinson et al., 2011, 2013). A simple, single, thermographic measure could provide similar diagnostic power to more complex clinical approaches. The thermographic apparatus employed in this work was relatively large and expensive. However, simpler, hand-held models are now available and at a cost which is affordable for potential use. The findings of the current work indicate that the application of portable thermography equipment for clinical diagnosis should be further explored.

7.6 References


Kallarackal GU, Ansari EA, Amos N, Martin JC, Lane C, Camilleri JP. A comparative study to assess the clinical use of Fluorescein Meniscus Time (FMT) with Tear Break up Time (TBUT) and Schirmer's tests (ST) in the diagnosis of dry eyes. Eye 2002; 16(5): 594-600.


Li W, Graham AD, Selvin S, Lin MC. Ocular Surface Cooling Corresponds to Tear Film Thinning and Breakup. Optom Vis Sci 2015; 92(9):e248-56.


8 Conclusions and Future Work

8.1 Summary and implications of key findings

The main purpose of this project was to study the diagnostic ability of IR ocular surface thermography in assessing dry eye disease (DED) and derive the important ocular temperature metrics for DED, as the conventional ways of diagnosing DED are problematic due to invasiveness, poor test reliability and time consuming.

The work started with a survey to describe the prevalence and risk factors of symptomatic dry eye disease (SDED) in Singapore (Chapter 3). The findings were required in the later part of the project. A cross-sectional dry eye survey was carried out using the McMonnies dry eye questionnaire (DEQ). Members of the public were interviewed at the 46 (out of 62) randomly selected mass rapid transit (MRT) stations and their vicinity. A total of 1004 questionnaires were collected for participants aged between 15 and 83 years old. Symptomatic dry eye disease (SDED) was defined as those with at least one of five self-reported symptoms that were reported as often or constantly. Non-dry eye (NDE) subjects were those with no related symptoms reported. Prevalence of SDED in the studied population and confidence interval (CI) were calculated. Risk factors were also evaluated using logistic regression analysis at 95% CI. The prevalence for SDED was found to be 12.3% with prevalence greater in females than males. SDED was shown to be significantly associated with contact lens wear (odds ratio [OR] 2.96, 95% CI: 1.81 – 4.83), those having had previous treatment for dry eye (OR 2.09, 95% CI: 1.33 – 3.29), those taking medication (OR 1.84, 95% CI: 0.99 – 3.44), those with unusual sensitivity of eyes (OR 3.04, 95% CI: 1.92 – 4.83), constant mucous membrane dryness (OR 4.11, 95% CI: 1.62 – 10.45), and irritation on waking (OR 2.38, 95% CI: 1.34 – 4.22). Smoking was not found to be associated with SDED. Singapore has SDED prevalence of 12.3% and was associated with contact lens wear, those had previous treatment in dry eye, medication, those having unusual sensitivity of eyes, mucous membrane dryness and waking irritation. These new data will be of value to the eye care community in Singapore and elsewhere.

Chapter 4 evaluated the repeatability of NEC infrared thermo-tracer TH9260 in assessing healthy and dry eyes. Ocular surface temperature (OST) was recorded using NEC infrared thermo-tracer TH9260 on 21 healthy and 15 dry eye subjects. Marking of the ocular surface and OST acquisition was performed using a novel ‘diamond’ demarcation method. Twelve OST indices were obtained at three different time points: 0 s, 5 s and 10 s. Repeatability of the infrared ocular thermography was evaluated in three aspects: (1) inter-image / image analysis repeatability; (2) inter-occasion / intra-examiner repeatability and (3) inter-examiner repeatability by calculating coefficients of repeatability (COR). Ten out of the twelve tested OST indices had good repeatability with small inter-image variability (%COR: 0.2 to 0.9), inter-occasion variability (%COR: 2.1 to 3.7) and inter-
examiner variability (%COR: 1.5 to 3.7) for the three studied time points. Two of the OST indices (temperature standard deviation of the region of interest and radial temperature difference) had poor repeatability with much larger inter-image variability (%COR: 8.9 to 140.7), inter-occasion variability (%COR: 47.5 to 153.5) and inter-examiner variability (%COR: 54.7 to 142.0) for the three studied time points. Most of the temperature metrics adopted in this assessment can be considered to be highly repeatable. This is the first study of the repeatability of NEC thermo-tracer TH 9260 in assessing both healthy and dry eyes, with twelve OST indices included in three different time points (0, 5 and 10 s). The findings of this study will determine the direction of future investigations on healthy and dry eyes (Tan et al., 2016).

Chapter 5 investigated static and dynamic measurement of OST on dry eyes. OST was again recorded using NEC TH9260 thermo-tracer on 62 dry eye patients and 63 age- and sex-matched controls and ocular surface marking/OST acquisition were done using the same ‘diamond’ method as described in chapter 4. Static measures were study of absolute OST at t = 0 s, 5 s and 10 s after eye opening. Dynamic measures were study of mean change and net change in OST over 10 s of sustained eye opening. Ten OST indices studied were: temperatures of the geometric center of the cornea (GCC), extreme temporal (T1) and nasal conjunctiva (T4), mid temporal (CT) and nasal conjunctiva (CN), temporal (LT) and nasal (LN) limbus, and mean ocular surface temperature (MOST), maximum (MaxT) and minimum (MinT) temperatures of the region of interest. For static measures, dry eye recorded a significantly lower GCC, MOST, MinT, MaxT, T4, CT, LT and LN as compared to controls at 0 s, 5 s and 10 s (one-way ANOVA, p < 0.05). The differences were highly significant (one-way ANOVA; p < 0.01) for GCC, MOST, MaxT, T4, CT and LT and were significant (one-way ANOVA; p < 0.05) for MinT and LN at 0 s, 5 s and 10 s. There were marginal significant differences found for CN at 5 s and 10 s and no significant differences found between the two groups for T1 at 0 s, 5 s and 10 s. For dynamic measures, dry eye had significantly steeper regression line of mean change (corresponding to greater net change) for MaxT from 5 s onward and T4 from 3 s onward. In conclusion, both static and dynamic measures of the OST were valuable and can be used as clinical tool to assess dry eye.

Chapter 6 examined the efficacy of IR ocular thermography in screening for DED. This is the first study to demonstrate that measuring temperature at the extreme nasal conjunctiva was able to discriminate mild to moderate dry eye from non-DED patients. The test was comparable to other well established methods in testing tear stability based on different principles. IR ocular thermography was performed using the same method on the same cohort as described in chapter 5. Thirty static- and thirty dynamic-metrics were generated from the ten OST indices used in chapter 5. Receiver operating characteristic curve (ROC) was plotted for each metric. The efficacy of the temperature metrics in diagnosing DED were evaluated singly and as combinations in terms of their area under the ROC curve (AUC), Youden index and discrimination power (DP). T4-5 and T4-10 (i.e. absolute temperature of the extreme nasal conjunctiva at 5 s and 10 s) were shown to be the best detectors for DED with good efficacy. When the cutoff values for T4-5 was set at 34.7
°C, sensitivity and specificity was 87.1% (95% CI: 76.2 to 94.3%) and 50.8% (95% CI: 37.9 to 63.6%) respectively. When the cutoff values for T4-10 was set at 34.5 °C, sensitivity and specificity was 77.6% (95% CI: 64.7 to 87.5%) and 61.9% (95% CI: 48.8 to 73.9%) respectively. The two metrics had moderate accuracy and limited performances with AUC of 72% (95% CI: 63 to 81%) and 73% (95% CI: 64 to 82%); Youden index of about 0.4 and DP of 1.07 and 1.05 respectively. None of the dynamic metrics was good detector for DED. Combining metrics was not able to increase the AUC. This work suggests some utility for the application of IR ocular thermography for evaluation of dry eye patients.

Chapter 7 investigated the diagnostic ability of various common conventional dry eye tests, their correlation with six T4 temperature metrics (T4-0, T4-5, T4-10, T4-A, T4-S and T4-GR) and derived the best composite/combined tests for DED. This was a single visit study included few common conventional tests for DED performed on 62 dry eye and 82 normal subjects: symptom evaluation using McMonnies DEQ (Mscore) and symptoms count (Scount), fluorescein break-up time (FBUT), corneal epithelial staining (CES) (National Eye Institute/Lemp’s scale), non-invasive break-up time (NIBUT) and tear meniscus height (TMH). Significant differences between dry eye and normal subjects demonstrated for Mscore, Scount, FBUT and CES (unpaired t-test, p < 0.0001) at 95% CI. NIBUT and TMH were not able to differentiate dry eye from normal subjects. No correlation (Pearson's coefficient, -0.203 to 0.209; p > 0.05) was found between Mscore, Scount, FBUT and CES with any of the temperature metrics. However, CES correlated significantly with TMH (r = 0.276; p = 0.030) and inversely correlated significantly with FBUT (r = -0.258; p = 0.043). Mscore has the best test performance in diagnosing DED, followed by Scount, FBUT and CES. Values of Mscore at 8 were found to give sensitivity of 87.1% (95% CI: 76.2 to 94.3%) and specificity of 92.7% (95% CI: 84.8 to 97.3%). Values of Scount at 1 were found to give sensitivity of 93.6% (95% CI: 84.3 to 98.2%) and specificity of 65.9% (95% CI: 54.6 to 76.0%). Values of FBUT at 2 s were found to give sensitivity of 58.1% (95% CI: 44.9 to 70.5%) and specificity of 87.8% (95% CI: 78.7 to 94.0%). Values of CES at grade 2 were found to give sensitivity of 71% (95% CI: 58.1 to 81.8%) and specificity of 59.8% (95% CI: 48.3 to 70.4%). Combining FBUT and CES with absolute extreme nasal conjunctival temperature at 10 seconds (T4-10) had successfully increased the AUC to 79% with sensitivity and specificity of 92.3% and 45.1% respectively. Combining CES with T4-10 (series) had shown almost similar AUC of 78% with sensitivity and specificity of 92.3% and 42.7% respectively. This work validated the efficacy of Mscore, Scount, FBUT and CES in diagnosing DED and further confirmed the discordance between signs and symptoms for DED. Combining CES with T4-10 (series) can be future objective tests for dry eye and can be applied in clinical setting.
8.2 Limitations of the study and suggestions for future work

This project has attempted to evaluate the diagnostic ability of IR ocular thermography with its important ocular temperature metrics and derive the best test (single or in combination) to be used in a clinical setting. There were several limitations in each of the studies which have already been outlined in respective chapters. The remainder of the present thesis chapter aims to highlight areas for future investigations with regards to sample size and subjects’ age, dry eye selection criterion, dry eye subtypes, dry eye severity, experimental methodology and the reliability of T4-10 in diagnosing DED, as a single test or when combine with CES.

In chapter 3, the prevalence of symptomatic DED in Singaporean was revealed as 12.3% limited to the studied age range of 15 to 83 years. As McMonnies DEQ was used as a tool to reveal risk factors, other possible factors could be omitted. For example, Meibomian gland disease/dysfunction (MGD) was not specifically evaluated in this study. MGD is one of the most common cause of SDED and is more prevalent in older patients (DEWS, 2007b). Factors such as omega-3 and omega-6 fatty acids (Miljanovic et al., 2005), connective tissue disease, LASIK and refractive excimer laser surgery (Hovanesian and Shah, 2011), radiation therapy, hematopoietic stem cell transplantation, vitamin A deficiency (Sommer, 2003), Hepatitis C infection (Zegans et al., 2002), androgen deficiency and diabetes mellitus (Kaiserman et al., 2005) that have been reported as possible risk factors for dry eye were not assessed. Future studies can be carried out using other questionnaire such as OSDI that reported to be able to grade severity and evaluate the impact on vision-related quality of life (Dougherty et al., 2011). The OSDI was found to correlate well with McMonnies DEQ and some other questionnaire studied (Schiffman et al., 2000) and unbiased towards the diagnosis of aqueous tear deficiency subtype of dry eye (Albietz, 2000). As members of the public were recruited at the MRT stations and nearby locations, the sample may not be representative of the Singapore population. This is because (1) not everyone takes MRT during the recruitment period and (2) the sample may be biased towards elderly peoples (that more prone to DED) who are not working during office hours. In addition, the demographic profiles i.e., the age/gender/ethnicity profiles of the study cohort should be correlated against the national data (Table 3.2 and 3.3). Currently the study cohort only have similar ethnicity profiling but not the age and gender profiles. A statistical analysis should be carried out as the age and gender profiles appear to be statistically different and recognised as one of the limitation of the work.

In chapter 4, measuring OST using NEC thermo-tracer TH9260 has shown to be repeatable in ten out of the twelve selected OST indices at three different time points: 0 s, 5 s, 10 s. Twelve OST indices were selected to document the whole inferior zone of the exposed ocular surface within the region of interest. Coefficient of repeatability and Bland-Altman plots were chose as indicators for repeatability in this study. Other methods would include study of inter- and intra-class coefficient and line of identity. The Bland-Altman plots provide graphical representation of the results. They show how the mean of the analysis / reanalysis OST was plotted against the difference between
the two measurements using the approach suggested by Bland and Altman (1986) and explore the relationship between measurement error and measurement magnitude and to derive the 95% limits of agreement. The results of the twelve OST indices were shown. Results section was written to shown details of the inter-image, inter-occasion and inter-examiner repeatability. As the three selected time points have always shown to give similar results, future studies may only consider one time point, for example 5 s as it reduces the tendency of reflex tears. 15 dry eye and 21 healthy subjects were studied and increasing the sample size may give more reliable results. It was initially attempted to analyse the two groups separately but results were found to be similar and therefore the two groups were combined. Even though the IR detector used in the current study had reasonably high resolution, marking of the anatomical structures of the ocular surface was still difficult. Using visible light image to superimpose with the infrared thermal image can be a possible strategy to overcome the limitation and serves as a mean for future research. In Table 4.3 to 4.5, paired t-test was used to study inter-image, inter-occasion and inter-examiner repeatability at each time point (0, 5 and 10 s) and the p values were presented. In addition to that, one-way ANOVA could also be carried out to study variability in results across the three time points.

Even though the thermo-tracer used in the current study had reasonably high resolution, marking of the anatomical structures of the ocular surface was still difficult. The difficulty/subjectivity of locating/marking the different anatomical features of interest, particularly the centre of the cornea and the nasal and temporal limbus was apparent. The idea of capturing a visible light image of the eyes of the patients and, through digital image analysis, overlapping both images, could help in locating these anatomical features. This was one of the limitations of the current study where future improvements are indicated. In addition, the instrument employed in this study does not provide significantly better thermo-images than those already described in the literature. It was therefore the novel ‘diamond’ method was developed for several reasons: (1) to overcome problems of the truncated image by upper lids reported previously (Pattmoller et al., 2014) and (2) to minimize possible inconsistency in OST acquisition due to variation in palpebral aperture size particularly in Asian eyes and (3) to enable study of the inferior zone of the ocular surface which was reported to be a predictive area in the detection of dry eye subtypes (Fenner and Tong, 2013) and is generally not obscured by eyelashes during ocular thermography.

In chapter 5, dry eye was shown to have cooler ocular surface as compared to age- and sex-matched controls. The possible reasons to that was (1) the ocular surface was cooled by a thinner tear film leading to lower temperature recorded on various ocular surface areas (geometric center of the cornea, conjunctiva and limbus). This has also caused a reduction in MOST, MinT and MaxT. Thinner tear film was as a result of thinner tear film lipid layer (TFLL) in dry eyes (Nichols et al., 2005; King-Smith et al., 2013). As TFLL is important in tear instability and tear evaporation (Craig and Tomlinson, 1997), a thinner TFLL has caused higher tear instability and evaporation rate in dry eye and as a result, a cooler ocular surface; (2) “cold receptors” that primarily been activated by small shifts in the temperature of the ocular surface as a results of tear evaporation
“Cold receptors” is a class of ion channels identified in nerve endings and in corneal and conjunctival epithelial cells that can mediate the pain transduction from the ocular surface (Belmonte and Gallar, 2011) and may induce ocular discomfort. It was clear that tear evaporation was one of the main reasons causing changes of the temperature as described in chapter 5 but the relationship between tear evaporation and OST has never been studied. On the other hand, a warmer overall ocular surface (reported as higher MOST) has also been reported and accounted by increased conjunctival hyperaemia (Morgan et al., 1995, Singh and Bhinder, 2005b) and higher blink rate (Tan et al., 2009) in dry eye. As discussed in chapter 5, the conflicting results was likely due to differences in dry eye selection criterion, dry eye subtypes and severity, subject age and experimental methodologies. Future studies can be done to further address this. In this project mild to moderate dry eye patients were examined. It was because (1) it is a more commonly presenting form of dry eye in clinical practice and (2) few reports in the literatures were on severe dry eye (Morgan et al., 1995, Kamao et al., 2011). If this was the first study on dry eye, it could have been done on severe dry eye. Because there are already a range of studies in this area, severe dry eye which could be easier to differentiate using ocular thermography was not chosen. A wider cross-section of dry eye subjects were chosen instead as if ocular thermography could detect some temperature differences in mild to moderate dry eye, it would be able to do the same for severe dry eye patients. Nevertheless, this work can be expanded to more severe cases in the future. It is understood that dry eye patients would have difficulty to hold their blink for 10 s due to shorter tear break-up time. In the study, 10 s was tested for any benefit over 5 s and 0 s. For static measures, OST in dry eyes was different from controls at most of the studied indices at 0 s; For dynamic measures, T4 changed significantly 3 s onward and MaxT 5 s onward. It was therefore suggested that 10 s is not required as it is hard for dry eye patients to keep their eyes open for 10 s without inducing reflex tearing and blinking.

In chapter 6, the ability of ocular thermography in diagnosing DED was evaluated in terms of their sensitivity, specificity and receiver operating characteristics (ROC) curves. Area under the ROC curve (AUC), cutoff values, discrimination power and Youden’s index were reported. Measuring T4-5 and T4-10 (i.e. absolute temperature of the extreme nasal conjunctiva at 5 s and 10 s) were able to diagnose dry eye in the studied population. T4-10 was more superior than T4-5 in view of higher AUC and Youden index and was selected as an important temperature metric in diagnosing DED. It was sufficient to perform the test singly as combined metrics was not successful to increase its performance. In summary, measuring temperature at the extreme nasal conjunctiva has shown to be able to discriminate mild to moderate dry eye from subjects with normal tear film with good efficacy. The test was comparable to other well established methods based on different principles. In this chapter, thirty static- and thirty dynamic-metrics was selected initially and narrowed down to one most important metric (T4-10) after careful evaluation. Future investigations can just focus on static or absolute temperature measurement that shown to have better efficacy in diagnosing DED as compared to dynamic metrics. This study has shown that studying conjunctival
temperature was more valuable than corneal temperature in diagnosing DED. These findings required further investigations. As this is an early attempt in this particular set up it was important to standardise everything (e.g., temp and humidity controlled room, 20 minutes of room acclimation) and this will reduce errors as much as possible in this early stage. Subsequent work will need to be carried out in normal clinic setting environment in future to understand how ocular thermography will work. Future research will be needed on the variation in temperature and humidity etc to get a full understanding of utility of ocular thermography in clinical practice. Again, in this study it was not attempted to stratify subjects according to severity. All subjects were mild to moderate dry eye (as they appear in the clinic) and it was treated as one group at this stage.

In chapter 7, correlation between T4 temperature metrics and conventional dry eye tests were investigated and the best composite tests for DED was derived. This work validated the effectiveness of Mscore, Scount, FBUT and CES in diagnosing DED and further confirmed the discordance between signs and symptoms for DED. Combining CES with T4-10 (series) was shown to be future objective tests for dry eye. Further investigations need to be carried out to validate this findings by experimenting on a newly recruited cohort of dry eye and control subjects as recommended by Bron et al. (2007). Studies can be carried out to (1) validate the ability of T4-10 as a stand-alone test for DED (T4-10: determination of a referent for dry eye diagnosis) and (2) work out an algorithm and validate the diagnostic ability of the recommended combined test (CES and T4-10) using newly recruited subjects. Limitations as described in chapter 7 needed to be addressed and are summarised as follow:-

(a) Avoid studying conventional dry eye tests that have been used in the dry eye inclusion criterion to avoid biasness. If it needed to, it will be best to standardize testing method and do the measurement on the same day and in the same testing environment.

(b) Classify the severity of dry eye patients in a more standardized manner using a composite disease severity index, derived from the Dry Eye Workshop severity scale (DEWS, 2007a) and/or using the recommendation from the ODISSEY European Consensus Group (Baudouin et al., 2014).

(c) Ocular thermography and conventional dry eye tests should be done simultaneously so as the tear film condition would not change between measurements.

In summary, IR ocular thermography has demonstrated an ability to diagnose DED by just measuring T4-10. Still images can be taken at 1 s interval and diagnose DED by looking at the reading of extreme nasal conjunctiva at 10 s. If patient has reflex tears at 10 s, taking the reading at 5 s could be an alternative. Combining CES with T4-10 (series) could be the future objective tests for dry eye in the clinical setting. It must be noted that as there is no gold standard for DED, the best diagnostic efficacy is that the classification of patients by any new test is compared with a potentially flawed standard (in this case the comparison is with the ophthalmological diagnosis based on conventional clinical tests). The problem will remain in the diagnosis of dry eye, as in many other diseases, until a gold standard is defined.
Due to lack of gold standard for DED diagnostic criteria, it was not surprise to observe high correlation (and high AUC) in symptom evaluation. There could be non-dry eye patients presented with similar symptoms due to other disease (neuropathic pain, etc). This has been discussed in Chapter 7. Another problem of diagnosis in dry eye was the difficulty to diagnose subtypes of DED. The subcommittee of the International Dry Eye Workshop (DEWS, 2007a) revised the definition of dry eye to be: ‘a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and may also be accompanied with inflammation of the ocular surface’. Although it is well established that there are different types of dry eye, it is not known if ocular thermography can differentiate between aqueous deficient and evaporative dry eye. In this study, the term 'dry eye' has therefore been used to describe all types of dry eye.

With advanced technology, ocular thermography can be made much more convenient through portable equipment which is less expensive than high-end devices. Moving forward, it is possible to design (1) a handy and less expensive thermo-tracer that is hand-held or to be attached to slit lamp biomicroscope and use it as part of clinical routine examination and (2) an app than can be downloaded by practitioners to their mobile devices to capture ocular thermogram anytime, anywhere and pick up DED in seconds.
9 References


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Kallarackal GU, Ansari EA, Amos N, Martin JC, Lane C, Camilleri JP. A comparative study to assess the clinical use of Fluorescein Meniscus Time (FMT) with Tear Break up Time (TBUT) and Schirmer's tests (ST) in the diagnosis of dry eyes. Eye 2002; 16(5): 594-600.


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McCarty CA, Bansal AK, Livingston PM, et al. The epidemiology of dry eye in Melbourne,


APPENDIX 1 – Chapter 1

Appendix 1.1. Characteristics and current tests for dry eye (adapted from DEWS, 2007a).
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<table>
<thead>
<tr>
<th>Test</th>
<th>Reference</th>
<th>Cut-off value</th>
<th>Sensitivity (%)</th>
<th>FPR (%)</th>
<th>Specificity (%)</th>
<th>PPV*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Questionnaires</td>
<td>† McMonnies (1987)</td>
<td>Any</td>
<td>98</td>
<td>3</td>
<td>97</td>
<td>85</td>
</tr>
<tr>
<td>PRT</td>
<td>† Patel (1998)</td>
<td>≤ 10 mm</td>
<td>86</td>
<td>17</td>
<td>83</td>
<td>47</td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>† Goren (1988)</td>
<td>Any</td>
<td>25</td>
<td>10</td>
<td>90</td>
<td>31</td>
</tr>
<tr>
<td>Schirmer I</td>
<td>† Lucca (1990)</td>
<td>&lt; 5 mm/5 min</td>
<td>25</td>
<td>10</td>
<td>90</td>
<td>31</td>
</tr>
<tr>
<td>Schirmer I</td>
<td>† Farris (1981)</td>
<td>&lt; 3 mm/5 min</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Schirmer I</td>
<td>† Van Bijsterveld (1969)</td>
<td>&lt; 5.5 mm/5 min</td>
<td>85</td>
<td>17</td>
<td>83</td>
<td>47</td>
</tr>
<tr>
<td>Schirmer I</td>
<td>† Vitali (1994)</td>
<td>&lt; 10 mm/5 min</td>
<td>83</td>
<td>32</td>
<td>68</td>
<td>31</td>
</tr>
<tr>
<td>F BUT</td>
<td>† Vitali (1994)</td>
<td>&lt; 10 s</td>
<td>72</td>
<td>38</td>
<td>62</td>
<td>25</td>
</tr>
<tr>
<td>NIBUT</td>
<td>† Mengher (1985)</td>
<td>&lt; 10 s</td>
<td>83</td>
<td>15</td>
<td>85</td>
<td>49</td>
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<tr>
<td>TMS-BUT</td>
<td>† Goto (2004)</td>
<td>&lt; 5 s</td>
<td>98</td>
<td>37</td>
<td>63</td>
<td>32</td>
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<tr>
<td>Evaporation Rate</td>
<td>† Khanal (2006)</td>
<td>33 g/m²/h</td>
<td>51</td>
<td>4</td>
<td>96</td>
<td>84</td>
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<tr>
<td>Meniscus Height</td>
<td>† Mainstone (1996)</td>
<td>≤ 0.35 mm</td>
<td>93</td>
<td>33</td>
<td>67</td>
<td>33</td>
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<tr>
<td>Meniscus Radius</td>
<td>† Yokoi (2004)</td>
<td>≤ 0.25 mm</td>
<td>89</td>
<td>22</td>
<td>78</td>
<td>42</td>
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<tr>
<td>Tear film index</td>
<td>† Xu (1995a)</td>
<td>≤ 95</td>
<td>67</td>
<td>40</td>
<td>60</td>
<td>23</td>
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<tr>
<td>Tear Turnover Rate</td>
<td>† Khanal (2006)</td>
<td>12 %/min</td>
<td>80</td>
<td>28</td>
<td>72</td>
<td>79</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>† Farris (1994)</td>
<td>&gt; 312 Mosm/L</td>
<td>95</td>
<td>6</td>
<td>94</td>
<td>73</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>† Tomlinson (2006)</td>
<td>&gt; 316 Mosm/L</td>
<td>69</td>
<td>8</td>
<td>92</td>
<td>60</td>
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<tr>
<td>Osmolarity</td>
<td>† Tomlinson (2006)</td>
<td>&gt; 316 Mosm/L</td>
<td>59</td>
<td>6</td>
<td>94</td>
<td>63</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>† Tomlinson (2006)</td>
<td>&gt; 312 Mosm/L</td>
<td>66</td>
<td>16</td>
<td>84</td>
<td>42</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>† Tomlinson (2006)</td>
<td>&gt; 322 Mosm/L</td>
<td>48</td>
<td>1</td>
<td>99</td>
<td>89</td>
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<td>317 Mosm/L</td>
<td>78</td>
<td>22</td>
<td>78</td>
<td>86</td>
</tr>
<tr>
<td>Osmolarity</td>
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<td>&gt; 318 Mosm/L</td>
<td>94</td>
<td>5</td>
<td>95</td>
<td>77</td>
</tr>
<tr>
<td>Lysozyme assay</td>
<td>† Van Bijsterveld (1969)</td>
<td>dia &lt; 21.5 mm</td>
<td>99</td>
<td>1</td>
<td>99</td>
<td>95</td>
</tr>
<tr>
<td>Ferning</td>
<td>† Norn (1994)</td>
<td>Area &lt; 0.06 mm²/µl</td>
<td>94</td>
<td>25</td>
<td>75</td>
<td>40</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>† Lucca (1990)</td>
<td>&lt; 90</td>
<td>35</td>
<td>30</td>
<td>70</td>
<td>17</td>
</tr>
<tr>
<td><strong>Combined Tests</strong></td>
<td></td>
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<tr>
<td><strong>(Parallel)</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sch + RB</td>
<td>† Farris (1994)</td>
<td>Any/ ≤ 1 mm/min</td>
<td>77</td>
<td>51</td>
<td>49</td>
<td>21</td>
</tr>
<tr>
<td>Sch + BUT</td>
<td>† Farris (1994)</td>
<td>&lt; 1 mm/min/ ≤ 105</td>
<td>78</td>
<td>44</td>
<td>56</td>
<td>24</td>
</tr>
<tr>
<td>Sch + BUT + RB</td>
<td>† Farris (1994)</td>
<td>&lt; 1 mm/min/ ≤ 105/Any</td>
<td>80</td>
<td>51</td>
<td>49</td>
<td>22</td>
</tr>
<tr>
<td>TTR + Evap + Osmol</td>
<td>† Khanal (2006)</td>
<td>&lt; 12%; &gt; 33; &gt; 317</td>
<td>100</td>
<td>34</td>
<td>66</td>
<td>81</td>
</tr>
<tr>
<td><strong>(Series)</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Sch + Osmol</td>
<td>† Farris (1994)</td>
<td>&lt; 1 mm/min; &gt; 312</td>
<td>25</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Lacto + Osmol</td>
<td>† Farris (1994)</td>
<td>&gt; 90; &gt; 312</td>
<td>35</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TTR + Evap + Osmol</td>
<td>† Khanal (2006)</td>
<td>&lt; 12%; &gt; 33; &gt; 317</td>
<td>38</td>
<td>0</td>
<td>100</td>
<td>100</td>
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<tr>
<td><strong>Discriminant function</strong></td>
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</tr>
<tr>
<td>Osmol + Evap + Lipid</td>
<td>† Craig (1995)</td>
<td>&lt; 0.4</td>
<td>96</td>
<td>13</td>
<td>87</td>
<td>56</td>
</tr>
<tr>
<td>TTR + Evap + Osmol</td>
<td>† Khanal (2006)</td>
<td>&gt; - 0.4</td>
<td>93</td>
<td>12</td>
<td>88</td>
<td>58</td>
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<tr>
<td>Symbols and abbreviations used in Appendix 1.1.</td>
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<td>-----------------------------------------------</td>
<td></td>
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<tr>
<td>* Assumes a dry eye prevalence of 15% in the population studies.</td>
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<tr>
<td>† Efficacy calculated in the sample from which the cutoffs were derived.</td>
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<tr>
<td>‡ Efficacy calculated in an independent sample of subjects.</td>
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<tr>
<td>§ Unpublished data</td>
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</table>

<table>
<thead>
<tr>
<th>Definition and abbreviations used in Appendix 1.1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUT Tear break-up time</td>
</tr>
<tr>
<td>Dia Diameter of the disc observed with the radial-immuno-diffusion Lactoplate method</td>
</tr>
<tr>
<td>Evap Tear film evaporation rate</td>
</tr>
<tr>
<td>F BUT Fluorescein tear breakup time</td>
</tr>
<tr>
<td>FPR False positive rate. The proportion of normals identified incorrectly as +ve by the test (Specificity is: 100-FPR)</td>
</tr>
<tr>
<td>Lacto Lactoferrin assay using the Lactoplate method</td>
</tr>
<tr>
<td>NIBUT Non-invasive tear breakup time</td>
</tr>
<tr>
<td>PPV Positive Predictive Value: the probability of truly having dry eye among those with a positive test result</td>
</tr>
<tr>
<td>PRT Phenol red thread test</td>
</tr>
<tr>
<td>RB Rose Bengal staining</td>
</tr>
<tr>
<td>Sensitivity Detection rate: the proportion of patients with disease who have a positive test result</td>
</tr>
<tr>
<td>Specificity Proportion of normal people with negative test result</td>
</tr>
<tr>
<td>TMS-BUT Tear break-up time measured with the Topographic Modeling System (Goto, 2004)</td>
</tr>
<tr>
<td>TTR Tear turnover rate, often measured with a scanning fluorophotometer (Fluorotron)</td>
</tr>
</tbody>
</table>
APPENDIX 2 – Chapter 2

Appendix 2.1a. DSRB ethics approval letter 1.

Appendix 2.1b. DSRB ethics approval letter 2 (extension).

Appendix 2.2. Approved DSRB application form.

Appendix 2.3. Subject information sheet and consent form.

Appendix 2.4. Record form.
Appendix 2.1a. DSRB ethics approval letter 1.

National Healthcare Group
Adding years of healthy life
DSRB Ref: A/10/390

13 September 2010

Dr Sanjay Srinivasan
Department of Ophthalmology
Khoo Teck Puat Hospital

Dear Dr Sanjay,

NHG DOMAIN SPECIFIC REVIEW BOARD (DSRB) APPROVAL

Project Title: Dry eyes in Singapore and ocular thermography as diagnostic tool for dry eyes

We are pleased to inform you that the NHG Domain Specific Review Board has approved the above research project to be conducted in Khoo Teck Puat Hospital.

Please note that this study can only be initiated after a Clinical Trial Certificate has been issued, or the Health Sciences Authority has given a written notification that a Clinical Trial Certificate is not required.

The documents reviewed are:

a) DSRB Application Form: Dry eyes in Singapore and ocular thermography as diagnostic tool for dry eyes, Version 1 dated 27 August 2010
b) Participant Information Sheet and Consent Form: Version 1 dated 05 August 2010
c) Recruitment Poster: Dry Eye Subjects Needed!, Version 1

The approval period is from 13 September 2010 to 18 July 2011. The reference number for this study is DSRB- A/10/390. Please use this reference number for all future correspondence.

Continued approval is conditional upon your compliance with the following requirements:

1. Only the approved Participant Information Sheet and Consent Form should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject should be given a copy of the signed consent form.

2. No deviation from, or changes of the protocol should be implemented without documented approval from the NHG DSRB, except where necessary to eliminate apparent immediate hazard(s) to the study subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g. change of monitor or telephone number).

1 of 2

203
DSRB Ref: A/10/390

3. Any deviation from, or a change of, the protocol to eliminate an immediate hazard should be promptly reported to the NHG DSRB within seven calendar days.

4. Please submit the following to the NHG DSRB:
   a. All unanticipated problems involving risk to subjects or others should be reported. In order to assist the DSRB, all reports should be accompanied by the NHG DSRB Unanticipated Problems Involving Risk to Subjects or Others Reporting Form. Please find all forms and guidelines on reporting on the internet at www.research.nhg.com.sg.
   b. Report(s) on any new information that may adversely affect the safety of the subject or the conduct of the study.
   c. NHG DSRB Project Status Report Form – this is to be submitted 4 to 6 weeks prior to expiry of the approval period. The study cannot continue beyond 18 July 2011 until approval is renewed by the NHG DSRB.
   d. Study completion – this is to be submitted using the NHG DSRB Project Status Report Form within 4 to 6 weeks of study completion or termination.

5. The NHG Research QA Program was launched in May 2006. The program aims to promote responsible conduct of research in a research culture with high ethical standards, and to identify potential systemic weaknesses and make recommendations for continual improvement. This research project may be randomly selected for completion of self assessment worksheet or for a study review by the QA team. For more information please visit www.research.nhg.com.sg.

Yours sincerely,

Dr Sim Kang
Chairman
NHG Domain Specific Review Board A

Cc: Institutional Representative, KTPH
    Departmental Representative of Ophthalmology, KTPH
Appendix 2.1b. DSRB ethics approval letter 2 (extension).

DSRB Ref: 2010/00390

18 July 2011

Dr Sanjay Srinivasan
Department of Ophthalmology
Khoo Teck Puat Hospital

Dear Dr Sanjay,

RENEWAL OF NHG DOMAIN-SPECIFIC REVIEW BOARD (DSRB) APPROVAL

PROTOCOL TITLE: Dry eyes in Singapore and ocular thermography as diagnostic tool for dry eyes

We are pleased to inform you that the NHG DSRB has renewed the approval for the above study, being conducted in Khoo Teck Puat Hospital. The approval period is from 18 July 2011 to 17 July 2012.

The documents reviewed are:

a) DSRB Study Status Report Form: SRF0001
b) Application Form: Version 1
c) Participant Information Sheet and Consent Form: Version 1 dated 03 August 2010
d) Recruitment Poster: Dry Eye Subjects Needed!, Version 1

Continued approval is conditional upon your compliance with the following requirements:

1. Only the approved Participant Information Sheet and Consent Form should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject should be given a copy of the signed consent form.

2. No deviation from, or changes of the protocol should be implemented without documented approval from the NHG DSRB, except where necessary to eliminate apparent immediate hazard(s) to the study subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g. change of monitor or telephone number).

3. Any deviation from, or a change of, the protocol to eliminate an immediate hazard should be promptly reported to the NHG DSRB within seven calendar days.

4. Please note that for studies requiring Clinical Trial Certificate, apart from the approval from DSRB, no deviation from, or changes of the Research Protocol and Participant Information Sheet
and Consent Form should be implemented without documented approval from the Health Sciences Authority unless otherwise advised by the Health Sciences Authority.

5. Please submit the following to the NHG DSRB:

a. All unanticipated problems involving human subjects including serious adverse events (SAE) should be reported. In order to assist the DSRB, all SAE reports should be accompanied by the NHG DSRB SAE Cover Note. Please find all forms and guidelines on reporting on the internet at www.research.nhg.com.sg.

b. Report(s) on any new information that may adversely affect the safety of the subject or the conduct of the study.

c. NHG DSRB Project Status Report Form – this is to be submitted 4 to 6 weeks prior to expiry of the approval period. The study cannot continue beyond 17 July 2012 until re-approved by the NHG DSRB.

d. Study completion or termination – this is to be submitted using the NHG DSRB Project Status Report Form within 4 to 6 weeks of study completion or termination.

6. The NHG Research QA Program was launched in May 2006. The program aims to promote responsible conduct of research in a research culture with high ethical standards, and to identify potential systemic weaknesses and make recommendations for continual improvement. This research project may be randomly selected for completion of self-assessment worksheet or for a study review by the QA team. For more information please visit www.research.nhg.com.sg.

Yours sincerely,

Dr Sam Kang
Chairman
NHG Domain Specific Review Board A

Cc: Institutional Representative, KTPH
Departmental Representative of Ophthalmology, KTPH

(This is a computer generated letter. No signature is required.)

DSRB – DOMAIN A MEMBERS LIST
(Term from April 2010 to March 2012)
Appendix 2.2. Approved DSRB application form.
A1 Please enter the full title for this study.
Dry eyes in Singapore and ocular thermography as diagnostic tool for dry eyes

A2 Study Administrators are persons who are responsible for administrative matters related to the Study. They can be the Study Coordinators, Research Nurses or Clinical Research Associates, and need not be part of the Study Team.

While the Principal Investigator remains the primary contact person, the DSRB may contact the Study Administrators for clarification of administrative matters related to the Study.

Study Administrators may also assist the PI in completing the various online forms and reports, however, only the PI may ‘submit’ these online forms and reports to the DSRB.

This section is optional but PIs are encouraged to nominate at least one Study Administrator. You may assign Study Administrators for this study below.
B1 Study Sites & Study Team Members

All investigators who have a responsibility for the consent process and/or direct data collection for this study should be listed below.

Study Team Members with registered user accounts with us will be notified of their participation in this study when the Application is submitted.

For a multi-centre study, please appoint a Site PI for each site (Mandatory).

The Principal Investigator will be the Site PI for their own Institution, and will also be the primary contact person for the DSRB.

(i) ‘Overall Principal Investigator’: Srinivasan Sanjay

(ii) Study Sites under the oversight of NHG DSRB (eg: NHG’s Institutions, St Luke’s Hospital, HSA, Dover Park Hospice, etc)

<table>
<thead>
<tr>
<th>No.</th>
<th>Study Site</th>
<th>Name</th>
<th>Study Role</th>
<th>Institution</th>
<th>Department</th>
<th>Min Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Khoo Teck Pu at Hospital - Alexandra Health Pte Ltd</td>
<td>NHG</td>
<td>yipcheeckhew Yip</td>
<td>Co-Investigator</td>
<td>Khoo Teck Pu at Hospital - Alexandra Health Pte Ltd</td>
<td>Ophthalmology</td>
</tr>
<tr>
<td>2</td>
<td>Khoo Teck Pu at Hospital - Alexandra Health Pte Ltd</td>
<td>Sanjay Srinivasan</td>
<td>PI</td>
<td>Khoo Teck Pu at Hospital - Alexandra Health Pte Ltd</td>
<td>Ophthalmology</td>
<td>Completed</td>
</tr>
</tbody>
</table>

(iii) Other external Study Sites under the supervision of the ‘Overall Principal Investigator’ (eg: Nursing Home, Community Hospitals, Public Community etc)

B2 External Study Site (for Institutions NOT under the oversight of NHG DSRB)

(i) Are there any other independent study sites by another PI which are conducting the same study?

☐ Yes

☐ SingHealth
☐ Other Local Sites

☐ Overseas Sites
☐ No

B3 Research Specialty
Please indicate the Primary Specialty.

<table>
<thead>
<tr>
<th>No.</th>
<th>Primary Specialty</th>
<th>Primary Sub Specialty</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ophthalmology</td>
<td>Age Related Macular Degeneration</td>
</tr>
</tbody>
</table>

Please indicate/add Secondary Specialties.

<table>
<thead>
<tr>
<th>No.</th>
<th>Primary Specialty</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Others</td>
<td>0</td>
</tr>
</tbody>
</table>

B4

i. Which Domain Specific Review Board (DSRB) is this application being submitted to? DSRB Domain A

ii. Has the study been submitted to another IRB?
- No
- Yes

iii. Has the application been previously rejected by any IRB? (Including NHG-DSRB)
- No
- Yes
Ethics Main Application Form (Section C - Conflict of Interest Declaration)

The Conflict of Interest Declaration section must be completed by the PI on behalf of the Study Team if any member of the Study Team has any potential conflicting interest while conducting the research. Any such member(s) must complete and submit their Declarations when this application is submitted.

Conflicting Interest - A conflicting interest can be broadly defined to refer to any interest of the investigator that competes with the investigator’s obligation to protect the rights and welfare of research subjects.

Financial Interest - Significant Financial Interest means anything of monetary value, including but not limited to, salary or payments for services (e.g. consulting fees or honoraria); equity interests (e.g. stocks, stock options or other ownership interests); intellectual property rights (e.g. patents, copyrights and royalties from such rights), and board or executive relationships.

The Conflict of Interest Declaration Section must be submitted to the DSRB via protocol amendments if any of the circumstances relevant described herein change during the conduct of the research.

Sanjay Srinivasan
- Yes
- No
Ethics Main Application Form (Section D - Nature of Research)

D Please select a category that best describes your application. Please note that for clinical trials, additional questions are applicable. Please take some time to choose carefully.

Clinical Trials
Choose this if your research involves:
(1) Administering a drug, device, or biologic as part of the research intervention, or
(2) Performing surgical procedures as part of research intervention

Questionnaire/ Survey/ Interviews
Choose this if your research involves:
(1) Administering questionnaires/surveys/interviews. This type of research may also include a medical records review component.

Medical Records Review
Choose this if your research involves:
(1) Collection of data for a specific research project by review of medical records including results of routine diagnostic tests performed for standard clinical purposes
(2) Prospective and/or retrospective data collection

Clinical Research
Choose this if your research involves:
(1) Collection of blood by venepuncture, finger stick, etc.
(2) Prospective collection of biological specimen by invasive or non invasive means including biopsies, FNAC’s, fundoscopy etc.
(3) Collection of data through research procedures such as X rays, MRI, ultrasound, ECG, EEG, etc.
(4) Any other research categories.

D1 Please select one category that best describes your research activities.
• Clinical Trials (which Includes Drug, Device and Surgical-Procedural Trials)
• Questionnaire/ Survey/ Interviews
• Medical Records Review
• Clinical Research

Note: Clinical Trial Certificate from Health Sciences Authority might be required if you are testing the safety and efficacy of the medicinal product. You should check with HSA if you are unsure.

D2 Is this a US FDA IND/IDE study or data is intended to be reported to FDA in support of a IND/IDE application?
• Yes
• No

Note: US FDA-regulated (IND) research activities cannot qualify for Exemption from DSRB Review and Waiver of Informed Consent. The application must be submitted using the Non-Exempt Application Form.

D3 Is this study subjected to any of the following regulations:
• No
• Yes

☐ Others
E1 Who will be responsible for the payment and compensation of injury or illness arising from participation of subjects in the study?

(Note: For investigator - For investigator-initiated studies - Contact your OBR/CRU for more information on available NHG Clinical Trial Compensation Insurance Scheme.)

Khoo Teck Puat Hospital Clinical Research Unit

E2 Please give information regarding the study’s Funding source or Sponsor information.
- □ No funding is required for this study to be carried out
- □ Pharmaceutical / Industry Sponsored
- ● Grant

i. Name of Grant Agency and Grant Name Others
   Please specify: Not Applicable (Non NHG)

ii. Grant amount applied for 108500.0

iii. Date of Grant application deadline 01-Jun-2011

iv. Has the Grant application been approved?
   ● Yes. Grant application successful.

   Date of Grant Approval: 01-Apr-2009
   Date of Grant Expiry: 01-Jun-2011
   Amount of Grant awarded: 108500.0

   Please attach the approved grant proposal and all relevant documents approved by the grant body(e.g. study protocol, consent form etc)

- □ No. Grant application is pending approval.

E3 Who will be responsible for research-related costs? For sponsored studies, please list the costs that will be borne by the sponsor. You may wish to attach the Financial Agreement / Clinical Trial Assurance if it is available.

The Clinical Research Unit of Khoo Teck Puat Hospital will bear the costs related to any injury which could arise as subjects participating in the study. The reimbursement cost for subjects participating in the study will be borne by the department of Optometry, Singapore Polytechnic. There won’t be any charges for a visit on a separate day.
F1 Please provide an abstract of your proposed research (Up to 300 words).
Your abstract must contain:
Aims
Methodology
Importance of proposed research to science or medicine
Potential benefits & risks

AIMS: To find an efficient and effective way to screen/diagnose dry eyes METHODOLOGY: 40 dry eye patients and 40 age- and sex-matched controls will be recruited. Subjects (masked from being dry eyes or controls) will be randomised and tested using (1) ocular thermography (2) high-speed videotopography and (3) the measurement of tear meniscus height on a slit lamp biomicroscope. The randomisation of subjects is for the order of the tests. Patient’s will be administered Mc monnie’s questionnaire which has queries on patient’s eye symptoms and medical history. INTROIMPORTANCE: Non invasive methods to diagnose dry eye is required. This study may fulfill that void. POTENTIAL BENEFITS: Participate in a study which helps in a non-invasive diagnosis of dry eye. New information obtained could help in better management of patient. POTENTIAL RISKS: None, non invasive diagnostic tests. Keep the lids open may cause subjects to experience some dryness in the eyes.

F2 What are the Specific Aims of this study?
To find an efficient and effective way to screen/diagnose dry eyes
To find a new efficient and effective way to screen/diagnose dry eyes

F3 What is the Hypothesis of this study?
To explore the role of ocular thermography/high-speed videotopography to diagnose and manage dry eyes.

F4 Please briefly describe the background to the current study proposal. Critically evaluate the existing knowledge and specifically identify the gaps that the proposed study is intended to fill.
Ocular thermography has the potential to diagnose and manage dry eyes. Ocular thermography: The tear film has been claimed to be about 40 μm and therefore all radiation which reaches the infrared detector must emanate from the tear film. Capturing the ocular surface temperature changes will reflect the changes on the tear film. As the tear film changes rapidly, it is therefore important to capture it real time using high resolution (640 x 480) IR thermal camera that can provide real-time video images (thermal video) with high frequency, 30 Hz (30 frames/second) with high sensitivity (0.06°C). Prior to thermography, subjects will be adapted to a room with controlled temperature and humidity for 20 minutes. Earlier work had established that this period was necessary to achieve ocular temperature stabilisation. None of the dry eye subjects will instil eye drops on the days of measurement. Prior to examination, subjects will be asked to blink several times, close their eyes for 3 seconds and then hold the eyes open for as long as possible. Dynamic mapping of ocular thermograms will be carried out for one eye. Time and position for 1st dry spot to appear will be noted. Various measures of ocular surface temperature will be assessed including absolute values, the variance of temperature across the ocular surface and the difference in temperature between the limbus and the centre of the cornea. The same procedures will be repeated on the other eye. High-speed videotopography: High-speed videotopography has the potential to provide new information on dynamic changes of corneal topography and tear film behaviour. Medmont £300 unit (Medmont Pty Ltd, Melbourne, Australia) will be able to acquire dynamic images on the eye at a rate of 50 Hz (that is, one frame every 20 milliseconds). With this, tear film stability can be analysed in the inter-blink interval the tear film build-up (time for tear film to reach the most regular state) and break-up times (time for tear film to rupture) can be measured. Subjects will be placed in front of the machine and asked to blink several times, hold the eyes for as long as he/she can and dynamic mapping of the tear film at different meridians will be acquired, one eye followed by the other. Again, time and position for 1st dry spot to appear will be noted. Tear break-up time measured with this will be termed as NITBUT (non-invasive tear break-up time). It is a qualitative measure of the tear stability. This method has been proven to be less variation as compared to the conventional fluorescein-instillation tear break-up time (TBUT) and it is also more ‘natural’. It has been recommended that five measures should be taken and averages the three closest measures as representative of tear stability. Mapping of the stability of tear film will be correlated
with the ocular thermography findings on the same subject. Corneal topography will also be measured as
blood supply and the curvature of ocular surface were two factors that affect the temperature of the eye. Tear
Meniscus Height (TMH) The “tear meniscus” are the two tear ‘rivers’ formed at the upper and lower lid margins.
The height of it is termed the tear meniscus height (TMH). It is a quantitative measure of the tear volume.
A CCD camera connected to a slit lamp biomicroscope and the Topcon IMGEnet system will be used and
calibrated to measure TMH. The slit will be set at 3x0mm vertical slit, 16x magnification, and illumination level
set at normal. Images of the tear meniscus within 5 mm temporal from the middle of lower lid margin will be
captured and stored. To avoid reflex tearing, a short light beam will be used to prevent direct shining of light
into pupil. This system has been proven to show specificity and sensitivity of 70% and 81.8% respectively in
tear analysis. Subjects will be asked to look straight ahead while their TMH of the lower eye lid is measured.
Due to gravity, the lower TMH is more stable and clinical more important compared to the upper TMH. All
subjects will be asked to come for TMH measure after 1pm and those who used eye drops or eye ointment
will be asked not to use them on the day of the measurement. Findings on the above mentioned three tests
will be carried out and masked among three independent examiners. All three examiners are well-trained and
qualified examiners. At the end of the session, data on dry eye and control subjects will be tabulated and
analysed using two-way ANOVA. A research paper will be written from this project. The results will provide
useful diagnostic information about ocular thermography and if it has the potential to screen/diagnose dry
eyes. To our best knowledge, such work has not been conducted in this region. Measurement (1) will be
carried out by two different examiners to study its reproducibility as well as at three different times in a day
(in between a 30 minutes break) to study its repeatability, on both dry eye subjects and controls.

Please provide a list of relevant references.

1) Purslow C, Wolffsohn JS. Ocular surface temperature. Contact Lens Association of Ophthalmologists, Inc.
   2005; 31(3):117-1232 Morgan PB, Tullo AB, Efron N. Infrared thermography of the tear film in dry eye. Royal
   College of Ophthalmologists 1995; 615-6183) Tan L, Cai ZQ, Lai NS. Accuracy and sensitivity of the dynamic
   ocular thermography and inter-subjects ocular surface temperature (OST) in Chinese young adults. Contact
   Lens amp; Anterior Eye 2005; doi: 10.1016/j.clae.

Please submit a copy of at least two relevant papers.

OST_a review.pdf
Ocular sf temp.pdf

Please state concisely the importance of the research described in this application by relating the specific
aims to the long term objectives.

Non invasive methods to diagnose dry eye is required. This study may fulfill that void.

Discuss in detail the experimental design and procedures to be used to accomplish the specific aims of
the study.

Ocular thermography High-speed videotopography Tear Meniscus Height (TMH) measurement The details
have been elucidated earlier.

Please provide details on sample size and power calculation and the means by which data will be analyzed
and interpreted (If applicable).

Case-control study of 40 subjects and dry eye patients. They will be age and sex matched. A confidence
interval of 2-18% with confidence level of 95% will yield a sample size of 38 subjects. A major factor
determining the length of confidence interval is the size of the sample used. In this study we have funding for
40 subjects each and hence the confidence intervals.

List all research related activities.

1) Prior to thermography, subjects will be adapted to a room with controlled temperature and humidity
   for 20 minutes. Earlier work had established that this period was necessary to achieve ocular temperature
   stabilisation. The room with controlled temperature is to eliminate the influence of environmental conditions
   affecting ocular surface temperature. Mapstone (1968) in his study reported that for every degree fall in
   the environmental temperature, there is a decrease of 0.145°C drop in corneal temperature. Our study
   the room temperature will be controlled at about 24 +/- 0.3°C and humidity of 50 +/- 0.8% measured using an
   instrument called hygrometer. This controlled temperature is similar to a normal air-conditioned room and
won't be particularly uncomfortable patients for people without any illness. None of the dry eye subjects will

be asked to blink several times, close their eyes for 3 seconds and then hold the eyes open for as long as possible. Dynamic mapping of
ocular thermograms will be carried out for one eye. Time and position for 1st dry spot to appear will be noted. Various measures of ocular surface temperature will be assessed including absolute values, the variance of temperature across the ocular surface and the difference in temperature between the limbus and the centre of the cornea. The same procedures will be repeated on the other eye. 2) Subjects will be placed in front of the
machine and asked to blink several times, hold the eyes for as long as he/she can and dynamic mapping of
the tear film at different meridians will be acquired, one eye followed by the other. Again, time and position for 1st dry spot to appear will be noted. Tear break-up time measured with this will be termed as NIBUT (non-invasive tear break-up time). It is a qualitative measure of the tear stability. This method has been proven
to be less variation as compared to the conventional fluorescein-Instillation tear break-up time (TBUIt) and it is also more 'natural'. It has been recommended that five measures should be taken and averages the three closest measures as representative of tear stability. Mapping of the stability of tear film will be correlated
with the ocular thermography findings on the same subject. Corneal topography will also be measured as
blood supply and the curvature of ocular surface were two factors that affect the temperature of the eye. 3) A
camera connected to a slit lamp biomicroscope and the Topcon IMAGEnet system will be used and calibrated
to measure Tear meniscus height (TMH). The slit will be set at 3x6mm vertical slit, 16x magnification, and
illumination level set at normal. Images of the tear meniscus within 5 mm temporal from the middle of lower
lid margin will be captured and stored. To avoid reflex tearing, a short light beam will be used to prevent direct
shining of light into pupil. This system has been proven to show specificity and sensitivity of 70% and 81.8% respectively in tear analysis. Subjects will be asked to look straight ahead while their TMH of the lower eye
lid is measured. Due to gravity, the lower TMH is more stable and clinical more important compared to the
upper TMH. All subjects will be asked to come for TMH measure after 1pm and those who used eye drops or
eye ointment will be asked not to use them on the day of the measurement. Findings on the above mentioned
three tests will be carried out and masked among three independent examiners. All three examiners are
well-trained and qualified examiners. At the end of the session, data on dry eye and control subjects will be
processed and analysed using two-way ANOVA. A research paper will be written from this project. The results
will provide useful diagnostic information about ocular thermography and if it has the potential to screen/
diagnose dry eyes. To our best knowledge, such work has not been conducted in this region. Measurement (1)
will be carried out by two different examiners to study its reproducibility as well as at three different times in a
day (in between a 30 minutes break) to study its repeatability, on both dry eye subjects and controls.

F11 List all activities that are performed for routine diagnostic or standard medical treatment purposes.
The ey examination of patients as per standard protocol like measuring visual acuity, intraocular pressure,
auto refraction and fundus evaluation.

F12 Please describe the subject's visits (frequency and procedures involved). For studies with multiple visits,
please attach study schedule. (If applicable)

Single visit for the research study

(1) Routine eye evaluation
(2) Ocular temperature measurement
(3) Tear film measurement

F13 Discuss the potential difficulties and limitations of the proposed procedures and alternative approaches to
achieve the aims.

Recruiting the patients as subjects for the study. Alternative approaches is to advertise using the information
poster.

F14 What are the Potential Risks to Subjects?
None, non invasive diagnostic tests. Keeping the lids open may cause subjects to experience some dryness in
the eyes.

F15 What are the Potential Benefits to Subjects?
Participate in a study which helps in a non-invasive diagnosis of dry eye. New information obtained could help
in better management of patient...
F16 Preliminary Studies / Progress Reports. Please provide an account of the Principal Investigator's preliminary studies (if any) pertinent to this application.

Our collaborator Ms Tan Li Li has done the same study in Department of Optometry, Singapore Polytechnic. Subjects were recruited (26 dry eyes and 31 controls, n=57) in Singapore Polytechnic. Preliminary results have been mentioned in this journal Tan L, Cai ZQ, Lai NS. Accuracy and sensitivity of the dynamic ocular thermography and inter-subjects ocular surface temperature (OST) in Chinese young adults. Contact Lens amp; Anterior Eye 2009; doi: 10.1016/j.clae.

F17 What is the estimated timeline for this study?

Estimated Start Date: 01-Aug-2010

Estimated End Date: 31-Jul-2011

F18 Does this study have a Study Protocol?

- Yes
- No

F19 The PI is responsible for ensuring that all Study Subjects give informed consent before enrolling into the study.

Please select all the applicable consent scenarios.
- Informed Consent will be taken for all study subjects.
- Waiver of Informed Consent is requested for all study subjects.
- A combination of both Informed Consent and Waiver of Consent is required for different study populations.
Ethics Main Application Form (Section H - Recruitment Details)

H1 How will potential subjects be identified? (Please tick all the applicable boxes)
- Referral by attending healthcare professional
- Patients of study team
- Databases
- Other methods of subject identification

H2 Who will make the first contact with subject (Enter NA if not applicable)?
The Site-PI/PI will make the first contact with the potential subjects for the study. The Ophthalmology department at Khoo Teck Puat Hospital will make suitable referrals to the PI/Site PI.

H3 How will the subject be contacted (Enter NA if not applicable)?
By poster advertisement and other Non-NHG collaborator referrals. Patients in the Ophthalmology department at Khoo Teck Puat Hospital will be contacted.

H4 Will any advertising / recruitment materials be used to recruit research subjects?
- Yes

Please tick all the applicable types of advertising / recruitment materials that will be used in this study.
- (i) Posters

Please state where the posters will be placed, and attach a copy of the poster:
Will be placed in various locations in the Department of Ophthalmology, Khoo Teck Puat Hospital.

DSRB_Dry eye poster_Aug 13.gif

- (ii) Brochures
- (iii) Advertisements in Newspapers / Magazines / Publications
- (iv) Advertisements on Radio / TV.
- (v) ‘Letter of Invitation’ to potential research participants. ‘Letter of Invitation’ refers to email, letters or any form of documents used as part of the recruitment strategy, with the intention of inviting the research participants to participate in the study.
- (vi) Letter to Doctors requesting for referrals.
- (vii) Other types of materials will be used.

- No

H5 Will any other recruitment strategies be used? (Eg. Talks in public places, societies etc.)
- Yes
- No

H6 What is the Recruitment Period (If applicable)? Please provide us with the approximate recruitment period.

Start Date: 01-Aug-2010
End Date: 31-Jul-2011

H7 Please indicate the length of time of the subject’s direct involvement in the study. E.g. For clinical visits, examinations etc. (If applicable)
Approximately 2 hours
Please state the target number of research subjects to be recruited for each study site in Singapore. If exact numbers are not available, please give an approximate number.

(For back to Section B1 to add additional study site)

<table>
<thead>
<tr>
<th>No.</th>
<th>Study Site</th>
<th>Recruitment Target Min</th>
<th>Recruitment Target Max</th>
<th>Males</th>
<th>Females</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Khoo Teck Puat Hospital - Alexandra Health Pte Ltd</td>
<td>0</td>
<td>0</td>
<td>32</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Not Applicable (Non-NHG)</td>
<td>80</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Is this study part of an international study?
- Yes
- No
K1 Please list the inclusion criteria for research subjects in this study.
- 40 dry eye patients and 40 age- and sex-matched controls
- Controls subjects (n = 40) - asymptomatic (no history or symptom of tear film disorder)
- Not using any medications
- Good general and ocular health
- Non contact lens wearer
- Absence of dry eyes
- Dry eye subjects (n = 40) - a positive score on the McMonnies questionnaire and satisfy the following criteria:
  - Ocular symptoms either foreign body sensation or dryness when they were not using any eye drops.
  - Presence of corneal staining (Lemp's scale: >3 out of 15)
- No other ocular disorders
- Non contact lens wearer

K2 Please list the exclusion criteria for research subjects in this study.
- Inability to participate in the study
- Ocular surface disorders preventing visualising/analysing the test results
- Past history of ocular surgery that involves conjunctival dissection
- Those who have dry eye treatment done earlier including punctal plugs
- Previous ocular trauma/surgery

K3 Please state the age group of the research subjects.
- Lower Age limit 30
- Lower Age option years
- Upper Age limit 55
- Upper Age option years

K4 Are there any recruitment restrictions based on the gender of the research subjects?
  - Yes
  - No

K5 Are there any recruitment restrictions based on the race of the research subjects?
  - Yes
  - No

K6 Do the potential research subjects have a dependent relationship with the study team (e.g. doctor-patient, employee-employer, head-subordinate, student-teacher, departmental staff relationship)?
  - Yes
  - No

Please describe the dependent relationship.
- They are families/relatives of students or staff of Singapore Polytechnic. They may be existing patients of Khoo Teck Puat Hospital. There won't be any conflict of interests. They need to meet the inclusion criteria to participate in the study. All subjects will be treated equally and wouldn't be discriminated.

K7 Does the study involve any of the vulnerable research participants?
  - Yes
  - No

K8 Does the study involve any of the following?
  - Inpatients
  - Outpatients
  - Healthy volunteers
  - Not applicable
Ethnic Minor Application Form (Section P - Consent Process - Consent obtained)

P YES. Informed consent will be obtained from potential Research Participants before enrollment into the study.

The PI is responsible for ensuring that all Research Participants give informed consent before enrolling into the study. Please describe the consent process below.

P1 When will the consent process take place with the potential Research Participant?
After the patient has expressed his desire to participate in the study, the PI/ site PI or his designated staff will take the consent.

P2 Where will the consent process take place with the potential Research Participant?
Consent will be taken in the Department of Ophthalmology, Khoo Teck Puat Hospital.

P3 Who will conduct the consent process with the potential Research Participant?
PI/ site PI or his designated staff will take the consent.

P4 Describe how the consent process described above (in consideration of the time and place where consent is taken, and the person taking consent) minimize the possibility of coercion or undue influence.
The potential subjects are explained about the potential study. They are explained about the non-invasive tests in the study and asked about their ability to participate in the study. If they agree, they are scheduled to come another day for the research. they are also paid a travelling allowance of SGD 50 from the grant obtained by the investigator from Singapore Polytechnic, Ms Tan Li Li.

P5 Do you anticipate a situation where obtaining informed consent from a potential Research Participant is not possible and informed consent will be taken from the legally acceptable representative (including spouse, parent, and guardian)?
- No
- Yes

P6 Describe provisions to protect the privacy of Research Participants, where 'privacy interests' refer to interests of individuals to be left alone, free from intrusion and comfort with the proposed settings.
- If the potential patients are not interested in the study. They are treated as per existing standard protocol for dry eye. Patient’s consent will be taken in a private, quiet room, subjects will be given sufficient time to consider for participating in the study. The private room will be separate unused consultation room.

P7 Besides the Consent document, will any other materials or documents be used to explain the study to potential Research Participants? (eg. scripts, handouts, brochures, videos, logs, etc.)
- No
- Yes

P8 Will research participants receive any monetary payments (including transportation allowances) or gifts for their participation in the study?
- No
- Yes

Please provide details of the gifts and payment (including the amount paid).

- SGD 50. If the subjects fail the inclusion criteria, no reimbursement will be given but a token of appreciation will be given (eg. a packet of biscuits/candies).

P9 Will consent be documented in the form of a written and signed Research Participant Information Sheet and Consent Form?
- Yes, all Research Participants will be given a copy of the Research Participant Information Sheet and Consent Form.

Please attach a copy of the Information Sheet and Consent Form.

- No, Consent will not be documented. (E.g. verbal consent).

P10 Consent Language
(i) Will the study enroll non English speaking subjects?
   ○ No
   ○ Yes

P11 Will the study be recruiting subjects under emergency situations, when prior consent of the subject is not possible, and the consent of the subject's legally acceptable representative, if present, should be requested?
   ○ Yes
   ○ No

P12 Do you have any additional comments regarding the Informed Consent process?
   ○ No
   ○ Yes

Please elaborate:

The rationale for excluding Non-English speaking subjects is for administrative purposes only. We feel that English speaking subjects have materials in English and the collaborator can explain the scientific terms better in English. We have also not made any brochures/posters in languages other than English.
R In general, to protect the Study Subject’s confidentiality, research data should be coded, and the links between the Subject’s identifiers and the codes should be stored separately from the research data.

R1 Coded / anonymous research data will be sent to the study sponsor, and therefore no research database will be created and stored in NHG?
- No
- Yes

R2 Will any part of the study procedures be recorded on audiotape, film/video, or other electronic medium?
- No
- Yes

Page 18
S1 Will any biological materials (such as blood or tissue) be used as part of the study? This includes both prospectively collected and existing biological materials.

- No
- Yes
<table>
<thead>
<tr>
<th>T1 Who performs the data and safety monitoring? If there is a Data Safety Monitoring Board (DSMB), please submit the charter of the DSMB.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site PI/collaborator from Singapore Polytechnic and or PI from KTPH will monitor. There is no charter.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2 When and what safety data is monitored (Enter NA if not applicable)?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient data is anonymised and coded so that access is only to site PI/collaborator from Singapore Polytechnic and PI.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T3 When and how is data integrity monitored?</th>
</tr>
</thead>
<tbody>
<tr>
<td>The collected data is anonymised and kept under lock and key and password protect on the computer of our collaborator Ms Tan Li Li of Singapore Polytechnic.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T4 What are the criteria for stopping the research?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inability to recruit subjects; Expiry of grant; Adverse events like malfunctioning of equipments; Patient unable to complete the study because of any underlying medical condition.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T5 How will the outcome of data and safety monitoring be communicated to the study sites?</th>
</tr>
</thead>
<tbody>
<tr>
<td>It will not be communicated. It will be collected by Ms Tan Li Li of Singapore Polytechnic.</td>
</tr>
</tbody>
</table>
Ethics Main Application Form (Section U - Principal Investigator's Curriculum Vitae)

If any one of the study team member's curriculum vitae does not appear on this list, the CV must be uploaded through the user’s profile.

<table>
<thead>
<tr>
<th>No.</th>
<th>Study Site</th>
<th>Name</th>
<th>Study Role</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Khoo Teck Puat Hospital - Alexandra Health Pte Ltd</td>
<td>NGYipcheeckew Yap</td>
<td>Co-Investigator</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Khoo Teck Puat Hospital - Alexandra Health Pte Ltd</td>
<td>Sanjay Srinivasan</td>
<td>PI</td>
<td></td>
</tr>
</tbody>
</table>
Your DSRB Application is now complete and ready for submission.

**Principal Investigator's Declaration**

I will not initiate this study until I have received approval notification from the DSRB and all applicable regulatory authorities.

I will not initiate any change in the study protocol without prior written approval from the DSRB, except when it is necessary to reduce or eliminate any immediate risks to the Research Participants. Thereafter, I will submit the proposed amendment to the DSRB and all applicable regulatory authorities for approval.

I will promptly report any unexpected or serious adverse events, unanticipated problems or incidents that may occur in the course of this study.

I will maintain all relevant documents and recognise that the DSRB staff and applicable regulatory authorities may inspect these records.

I understand that failure to comply with all applicable regulations, institutional and DSRB policies and requirements may result in the suspension or termination of this study.

I declare that there are no existing or potential conflicts of interest for any of the investigators participating in this study.

By checking the "I agree" box, you confirm that you have read, understood and accept the Principal Investigator’s Declaration.

☐ I have read and agree to the above declaration.

Principal Investigator: Srinivasan Sanjay
Appendix 2.3. Subject information sheet and consent form.

PARTICIPANT INFORMATION SHEET

1. Study Information

Protocol Title:
Dry eyes in Singapore and ocular thermography as diagnostic tool for dry eyes

Site Principal Investigator & Contact Details:
Dr Yip Chee Chew,
Head and Senior consultant, Department of Ophthalmology and Visual Sciences,
Khoo Teck Puat Hospital, 90, Yishun Central, Singapore 768828
Tel :66023283
Email: yip.chee.chew@alexandrahealth.sg

Ms Tan Li Li, Singapore Polytechnic Optometry Centre,
W115, 500 Dover Road, Singapore 13965 (Tel: 68790493,
Email: tanlili@sp.edu.sg)

2. Purpose of the Research Study

You are invited to participate in a research study. It is important to us that you first take time to read through and understand the information provided in this sheet. Nevertheless, before you take part in this research study, the study will be explained to you and you will be given the chance to ask questions. After you are properly satisfied that you understand this study, and that you wish to take part in the study, you must sign this informed consent form. You will be given a copy of this consent form to take home with you.

You are invited because you have been selected as a control/case for dry eye( strike whichever is applicable)

This study is carried out to find out newer non-invasive diagnostic modalities for diagnosis of dry eye.

This study will recruit 40 subjects from Khoo Teck Puat Hospital over a period of 1 year from August 2010 to July 2011. About 26 dry eye patients and 31 controls were enrolled in a similar study conducted at Singapore polytechnic which was similar to this study.

3. What procedures will be followed in this study

Your participation in the study will last 2 hours and only for one visit.
If you agree to take part in this study, the following will happen to you:
You will be given a consent form to be completed.
You will be asked questions about your eye and general health.
You will be asked to fill up a questionnaire or asked on a number of questions stated on a questionnaire related to dry eye. It will only take about 5-10 minutes.

Followed which, you will be invited to participate in the second part of the study that involves the following measurements on your eyes (all will not cause harm to your eyes):

a) Sitting in front of a slit lamp biomicroscope where the status of eye health will be checked. A yellow staining agent (fluorescein dye) will be inserted into your eyes for the checking. This staining agent is does not cause any side effects except mild irritation in the eyes. After that, your tear river height will be measured.

b) Sitting in front of a high speed videokeratoscope where you will be asked to blink several times and hold as long as possible to measure the tear film as well as your eye profile.

c) Sitting in front of a thermal camera where you will be asked to blink several times and hold as long as possible to measure the change in your eye temperature. Your upper eye-lid will be lifted for the measurement. Besides, your body temperature will also be measured.

Three examiners will be taking the above measurements. All three examiners are well-trained and qualified examiners. If by any means a test is carried out by a student, he/she will be supervised by the principal investigator.

Only for measurement (c ), it will be repeated by another examiner and over three times on the same day, in between a 30 minutes break.

4. Your Responsibilities in This Study

If you agree to participate in this study, you should follow the advice given to you by the study team. You should be prepared to visit the hospital once and undergo all the procedures that are outlined above.

The study is being conducted because ocular thermography and videotopography are not yet proven to be a standard investigation in subjects with dry eyes. We hope that your participation will help us to determine whether these investigations are equal or superior to existing methods of diagnosis of dry eye

Although the investigations may not be part of standard medical care, in this study this/these procedure(s) are only being performed for the purposes of the research, and are not part of your routine care.

6. Possible Risks and Side Effects

There will be very minimal risk in all the above mentioned measurements. There may be discomfort of keeping the eyelids open forcibly for performing the tests. You may feel slight irritations/discomforts when the fluorescein staining agent is inserted onto your eyes. It will only temporary as the sensations will disappear by itself within few seconds.

Allergic reactions in the eye can occur with it albeit very rarely like itchiness, irritation, swollen lids. If you have any of these symptoms, call your doctor at once.

7. Possible Benefits from Participating in the Study
There is no direct benefit. However, your participation will contribute to the medical knowledge about dry eye prevalence and the use of ocular thermography in detecting dry eyes.

8. Alternatives to Participation

If you choose not to take part in this study, you will receive standard care for your condition. In our institution this would be the standard treatment protocol for the dry eye.

9. Costs & Payments if Participating in the Study

If you take part in this study, the following will be performed at no charge to you:

- Eye evaluation (Visual acuity, Intraocular pressure measurement, Slit lamp examination and retinal evaluation)
- Body temperature measurement
- Eye contour mapping for surface abnormalities
- Dry eye evaluation including tear break up time, tear quantity

You will be reimbursed for your time, inconvenience and transportation costs as follows:

- If you complete the study, you will be paid SGD 50
- If you do not complete the study for any reason, you will not be paid.

10. Voluntary Participation

Your participation in this study is voluntary. You may stop participating in this study at any time. Your decision not to take part in this study or to stop your participation will not affect your medical care or any benefits to which you are entitled. If you decide to stop taking part in this study, you should tell the Principal Investigator.

If you withdraw from the study, you won't be required to do anything.

Your doctor, the Investigator and/or the Sponsor of this study may stop your participation in the study at any time if they decide that it is in your best interests. They may also do this if you do not follow instructions required to complete the study adequately. If you have other medical problems or side effects, the doctor and/or nurse will decide if you may continue in the research study. In the event of any new information becoming available that may be relevant to your willingness to continue in this study, you (or your legally acceptable representative, if relevant) will be informed in a timely manner by the Principal Investigator or his/her representative.

11. Compensation for Injury

If you follow the directions of the doctors in charge of this study and you are physically injured due to the trial substance or procedure given under the plan for this study, the Clinical Research Unit, Khoo Teck Puat Hospital will pay the medical expenses for the treatment of that injury.

Payment for management of the normally expected consequences of your treatment will not be provided by the Clinical Research Unit, Khoo Teck Puat Hospital.
Clinical Research Unit, Khoo Teck Puat Hospital without legal commitment will compensate you for the injuries arising from your participation in the study without you having to prove Khoo Teck Puat Hospital is at fault. There are however conditions and limitations to the extent of compensation provided. You may wish to discuss this with your Principal Investigator.

By signing this consent form, you will not waive any of your legal rights or release the parties involved in this study from liability for negligence.

12. Confidentiality of Study and Medical Records

Information collected for this study will be kept confidential. Your records, to the extent of the applicable laws and regulations, will not be made publicly available.

However, NHG Domain-Specific Review Board and Ministry of Health will be granted direct access to your original medical records to check study procedures and data, without making any of your information public. By signing the Informed Consent Form attached, you (or your legally acceptable representative, if relevant) are authorizing such access to your study and medical records.

Data collected and entered into the Case Report Forms are the property of Khoo Teck Puat Hospital/Singapore Polytechnic. In the event of any publication regarding this study, your identity will remain confidential.

13. Who To Contact if You Have Questions

If you have questions about this research study, you may contact the Principal Investigator, Dr Yip Chee Chew,

Head and Senior consultant, Department of Ophthalmology and Visual Sciences, Khoo Teck Puat Hospital, 90, Yishun Central, Singapore 768828
Tel: 66023283
Email: yip.chee.chew@alexandrahealth.sg

Ms Tan Li Li, Singapore Polytechnic Optometry Centre, W115, 500 Dover Road, Singapore 13965 (Tel: 68790493, Email: tanlili@sp.edu.sg)

In case of any injuries during the course of this study, you may contact the Principal Investigator,

Dr Yip Chee Chew,
Head and Senior consultant, Department of Ophthalmology and Visual Sciences, Khoo Teck Puat Hospital, 90, Yishun Central, Singapore 768828
Tel: 66023283
Email: yip.chee.chew@alexandrahealth.sg
Ms Tan Li Li, Singapore Polytechnic Optometry Centre, W115, 500 Dover Road, Singapore 13965 (Tel: 68790493,
Email: tanlili@sp.edu.sg)

The study has been reviewed by the NHG Domain Specific Review Board (the central ethics committee) for ethics approval.

If you want an independent opinion of your rights as a research subject you may contact the NHG Domain Specific Review Board Secretariat at 6471-3266.

If you have any complaints about this research study, you may contact the Principal Investigator or the NHG Domain Specific Review Board Secretariat.
CONSENT FORM

Protocol Title:
Dry eyes in Singapore and ocular thermography as diagnostic tool for dry eyes

Principal Investigator & Contact Details:
Dr Yip Chee Chew,
Head and Senior consultant, Department of Ophthalmology and Visual Sciences,
Khoo Teck Puat Hospital, 90, Yishun Central, Singapore 768828
Tel :66023283
Email: yip.chee.chew@alexandrahealth.sg
Ms Tan Li Li, Singapore Polytechnic Optometry Centre,
W115, 500 Dover Road, Singapore 13965 (Tel: 68790493,
Email: tanlili@sp.edu.sg)

I voluntarily consent to take part in this research study. I have fully discussed and understood the purpose and procedures of this study. This study has been explained to me in a language that I understand. I have been given enough time to ask any questions that I have about the study, and all my questions have been answered to my satisfaction.

_______________________ _________________
Name of Participant Signature Date

Translator Information
The study has been explained to the participant / legally acceptable representative in ___ by ________________

Witness Statement
I, the undersigned, certify to the best of my knowledge that the participant signing this informed consent form had the study fully explained in a language understood by him / her and clearly understands the nature, risks and benefits of his / her participation in the study.

_______________________ _________________
Name of Witness Signature Date

Investigator Statement
I, the undersigned, certify that I explained the study to the participant and to the best of my knowledge the participant signing this informed consent form clearly understands the nature, risks and benefits of her participation in the study.

_______________________ _________________
Name of Investigator / Person administering consent Signature Date
Appendix 2.4. Record form.

<table>
<thead>
<tr>
<th>Personal Particulars</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Name:</td>
<td>Gender: M/F</td>
<td></td>
</tr>
<tr>
<td>Race: Chinese / Malay / Indian / Others</td>
<td>Age:</td>
<td>Room Temperature / Humidity:</td>
</tr>
<tr>
<td>Body Temperature:</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>History</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Itchiness □</td>
<td>Dryness □</td>
<td>Redness □ Tearing □</td>
</tr>
<tr>
<td>Surgeries □</td>
<td>Injuries □</td>
<td>Ocular diseases □ Ocular infections □</td>
</tr>
<tr>
<td>Diabetes □</td>
<td>Hypertension □</td>
<td>High Blood Pressure □ Smoking □</td>
</tr>
<tr>
<td>Others (Please specify):</td>
<td>Use of eye drops to relieve dry eye? Yes □ No □</td>
<td></td>
</tr>
<tr>
<td>Medications (Please specify):</td>
<td>Name of eye drop:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assessment</th>
<th>OD</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual Acuity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Distance)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slit Lamp:</td>
<td>![Slit Lamp Image]</td>
<td>![Slit Lamp Image]</td>
</tr>
<tr>
<td>Corneal Staining:</td>
<td>![Corneal Staining Image]</td>
<td>![Corneal Staining Image]</td>
</tr>
<tr>
<td>(Lemp Scale)</td>
<td>Grade 0</td>
<td>Grade 1</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>Grade 3</td>
</tr>
<tr>
<td>Position of 1st dry</td>
<td>![Position of 1st dry spot Image]</td>
<td>![Position of 1st dry spot Image]</td>
</tr>
<tr>
<td>spot (Draw location)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBUT</td>
<td>![TBUT Image]</td>
<td>![TBUT Image]</td>
</tr>
<tr>
<td>Ophthalmoscopy:</td>
<td>Es1[1(§)]</td>
<td>Es2[2(§)]</td>
</tr>
<tr>
<td>Ocular Thermography:</td>
<td>Es1(3§)</td>
<td>Es2(2§)</td>
</tr>
<tr>
<td>(Sign once taken)</td>
<td>Es1(1§)</td>
<td>Es2(3§)</td>
</tr>
<tr>
<td>Tear Meniscus Height (TMH):</td>
<td>![Tear Meniscus Height Image]</td>
<td>![Tear Meniscus Height Image]</td>
</tr>
<tr>
<td></td>
<td>![NIBUT Image]</td>
<td>![NIBUT Image]</td>
</tr>
</tbody>
</table>

Singapore Polytechnic
School of Chemical & Life Sciences – Optometry Department
APPENDIX 3 – Chapter 3

Appendix 3.1. McMonnies Dry Eye Questionnaire.
Appendix 3.2. DEWS dry eye diagnostic template (DEWS, 2007b).
Appendix 3.1. McMonnies Dry Eye Questionnaire.

<table>
<thead>
<tr>
<th>Personal Particulars</th>
<th>Race: Chinese / Malay / Indian / Others</th>
<th>Gender: M / F</th>
<th>Age: Under 25 / 25 – 45 / over 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you smoke?</td>
<td>☐ Yes</td>
<td>☐ No</td>
<td>☐ Yes</td>
</tr>
<tr>
<td>Have you worn contact lenses within the past 2 years?</td>
<td>☐ Soft contact lenses</td>
<td>☐ Hard contact lenses</td>
<td>☐ No contact lenses</td>
</tr>
<tr>
<td>1. Have you ever had drops prescribed or other treatment for dry eye?</td>
<td>☐ Yes</td>
<td>☐ No</td>
<td>☐ Uncertain</td>
</tr>
<tr>
<td>2. Do you ever experience any of the following eye symptoms? (can select more than one)</td>
<td>☐ Soreness</td>
<td>☐ Scratchiness</td>
<td>☐ Dryness</td>
</tr>
<tr>
<td>3. How often do your eyes have these symptoms?</td>
<td>☐ Never</td>
<td>☐ Sometimes</td>
<td>☐ Often</td>
</tr>
<tr>
<td>4. Are your eyes unusually sensitive to cigarette smoke, smog, air conditioning, central heating?</td>
<td>☐ Yes</td>
<td>☐ No</td>
<td>☐ Sometimes</td>
</tr>
<tr>
<td>5. Do your eyes easily become very red and irritated when swimming in chlorinated fresh water?</td>
<td>☐ Not applicable</td>
<td>☐ Yes</td>
<td>☐ No</td>
</tr>
<tr>
<td>6. Are your eyes dry and irritated the day after drinking alcohol?</td>
<td>☐ Not applicable</td>
<td>☐ Yes</td>
<td>☐ No</td>
</tr>
<tr>
<td><strong>Current Medication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>7. Do you take:</strong></td>
<td>(can select more than one)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Antihistamine tablets</td>
<td>□ Antihistamine eye drops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Diuretics (fluid tablets)</td>
<td>□ Sleeping tablets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Tranquillizers</td>
<td>□ Oral contraceptives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Medication for duodenal ulcer</td>
<td>□ Medication for digestive problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Medication for high blood pressure</td>
<td>□ None of the above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Please record any unlisted medication below:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>______________________________________</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>8. Do you suffer from arthritis?</strong></td>
<td>□ Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ No</td>
<td>□ Uncertain</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>9. Do you experience dryness of the mouth, nose, throat, chest or vagina?</strong></td>
<td>□ Never</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Sometimes</td>
<td>□ Often</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Constantly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>10. Do you ever suffer from thyroid abnormality?</strong></td>
<td>□ Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ No</td>
<td>□ Uncertain</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>11. Are you known to sleep with your eyes partly open?</strong></td>
<td>□ Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ No</td>
<td>□ Uncertain</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>12. Do you have eye irritation as you wake from sleep?</strong></td>
<td>□ Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ No</td>
<td>□ Uncertain</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Courtesy from Charles W. McMonnies, MSc UNSW, ASTC*
## Appendix 3.2. DEWS dry eye diagnostic template (DEWS, 2007b)

<table>
<thead>
<tr>
<th>DEWS</th>
<th>DRY EYE: DIAGNOSTIC TEST TEMPLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAPPORTEUR</td>
<td>Barbara Caffery</td>
</tr>
<tr>
<td>TEST</td>
<td>McMonnies questionnaire</td>
</tr>
<tr>
<td>TO DIAGNOSE</td>
<td>Presence or absence of dry eye</td>
</tr>
<tr>
<td>VERSION of TEST</td>
<td>[V2]</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>Mc Monnies 1987</td>
</tr>
</tbody>
</table>

### DESCRIPTION

The test is used to screen patients for the possibility of dry eye disease so that the index of suspicion of the practitioner is raised for those at risk and therefore further testing would be performed.

### CONDUCT of TEST

The test is self administered:
A questionnaire with 14 questions is given to the patient to fill out. The weighted values for questions are as follows.

- **Previous treatment** of dry eye:
  - yes = 2,
  - no = 0,
  - uncertain = 1

- **Experience of symptoms**: the presence of each symptom = 1
  - never = 0,
  - sometimes = 1,
  - often = 2,
  - constantly = 3

- **Unusual sensitivity of the eyes**:
  - yes = 2,
  - no = 0,
  - sometimes = 1

- **Swimming irritation of the eyes**:
  - yes = 2,
  - no = 0,
  - sometimes = 1

- **Alcohol use**: yes = 2, no = 0, sometimes = 1

- **Medication side effects**: each medication = 1

- **Arthritis**: yes = 2, no = 0, uncertain = 1

- **Mucous membrane dryness**:
  - never = 0,
  - sometimes = 1,
  - often = 2,
  - constantly = 3

- **Thyroid abnormality**:
  - yes = 2,
  - no = 0,
  - uncertain = 1

- **Nocturnal lagophthalmos**:
  - yes = 2,
  - no = 0,
  - uncertain = 1

- **Waking irritation**:
  - yes = 2,
  - no = 0,
  - uncertain = 1
Web Video: NA

Materials: A single sheet of paper with the questionnaire on it that includes the weighted scores.

Variations of technique: Some practitioners may not use the scoring system but just use the answers directly, in their decision making.

Diagnostic value: This version [v2]: [1987] To discriminate between normals and sicca syndrome. See below for sensitivity
Other version [V1]: [1986] Not as good on its own at identifying marginal dry eye.


Repeatability: Intra-observer agreement. [ ]
Inter-observer agreement. [ ]

Sensitivity: (true positives) [98%]

Specificity: (100 – false positives) [97%]

Other Stats: Mc Monnies 1986 refers to a different weighting system for the same questionnaire that was used to discriminate marginal dry eye from normals and more severe dry eye. The authors determined that neither history nor biomicroscopy alone were adequate to determine marginal dry eye. However, using the history to identify the top 10% of total scores, a high level of sensitivity was obtained.

Test problems: The questionnaire is not good at categorizing the patients as mild, moderate or severe.

References


The McMonnies questionnaire:

Please answer the following by underlining the response most appropriate to you.

Age: under 25 years 25-45 years over 45 years

Currently wearing: no contact lenses hard contact lenses soft contact lenses
1. Have you ever had drops prescribed or other treatment for dry eye?
   Yes (2) No (0) Uncertain (1)

2. Do you ever experience any of the following symptoms? (Please underline those that apply to you)
   1. soreness (1) 2. scratchiness (1) 3. dryness (1) 4. grittiness (1)
   burning (1)

3. How often do your eyes have these symptoms? (Underline)
   Never (0) Sometimes (1) Often (2) Constantly (3)

4. Do you regard your eyes as being unusually sensitive to cigarette smoke, smog, air conditioning, central heating?
   Yes (2) No (0) Sometimes (1)

5. Do your eyes easily become very red and irritated when swimming in chlorinated fresh water?
   Not applicable Yes (2) No (0) Sometimes (1)

6. Are your eyes dry and irritated the day after drinking alcohol?
   Not applicable Yes (2) No (0) Sometimes (1)

7. Do you take (please underline) antihistamine tablets (1), antihistimine eye drops(1), diuretics (fluid tablets) (1), sleeping tablets (1), tranquilizers (1), oral contraceptives (1), medication for duodenal ulcer (1) or digestive problems (1) or for high blood pressure (1) or __________ (1)

8. Do you suffer from arthritis?
   Yes (2) No (0) Uncertain (1)

9. Do you experience dryness of the nose, mouth, throat, chest or vagina?
   Never (0) Sometimes (1) Often (2) Constantly (3)

10. Do you suffer from thyroid abnormality?
    Yes (2) No (0) Uncertain (1)

11. Are you know to sleep with your eyes partly open?
    Yes (2) No (0) Uncertain (1)

12. Do you have eye irritation as you wake from sleep?
    Yes (2) No (0) Uncertain (1)
APPENDIX 4 – Chapter 4

Appendix 4.1. OST Analysis Program V2 written using MatLab Simulink 7.11.0 (R2010b).
Appendix 4.1. OST Analysis Program V2 written using MatLab Simulink 7.11.0 (R2010b).

```
function varargout = OSTAnalysis(varargin)
% OSTANALYSIS M-file for OSTAnalysis.fig
% OSTANALYSIS, by itself, creates a new OSTANALYSIS or raises the existing
% singleton*.
% H = OSTANALYSIS returns the handle to a new OSTANALYSIS or the handle to
% the existing singleton*.
% OSTANALYSIS('CALLBACK', hObject, eventdata, handles,...) calls the local
% function named CALLBACK in OSTANALYSIS.M with the given input arguments.
% OSTANALYSIS('Property','Value',...) creates a new OSTANALYSIS or raises the
% existing singleton*. Starting from the left, property value pairs are
% applied to the GUI before OSTAnalysis_OpeningFcn gets called. An
% unrecognized property name or invalid value makes property application
% stop. All inputs are passed to OSTAnalysis_OpeningFcn via varargin.
% *See GUI Options on GUIDE's Tools menu. Choose "GUI allows only one
% instance to run (singleton)".
% See also: GUIDE, GUIDATA, GUIN_HANDLES
% Edit the above text to modify the response to help OSTAnalysis
% Last Modified by GUIDE v2.5 29-Aug-2011 10:42:19
% Begin initialization code - DO NOT EDIT
gui_Singleton = 1;
gui_State = struct('gui_Name', mfilename, ...
   'gui_Singleton', gui_Singleton, ...
   'gui_OpeningFcn', @OSTAnalysis_OpeningFcn, ...
   'gui_OutputFcn', @OSTAnalysis_OutputFcn, ...
   'gui_LayoutFcn', [], ..., ...
   'gui_Callback', []);
if nargin && ischar(varargin{1})
gui_State.gui_Callback = str2func(varargin{1});
end
if nargout
   [varargout{1:nargout}] = gui_mainfcn(gui_State, varargin{:});
else
   gui_mainfcn(gui_State, varargin{:});
end% End initialization code - DO NOT EDIT

% --- Executes just before OSTAnalysis is made visible.
function OSTAnalysis_OpeningFcn(hObject, eventdata, handles, varargin)
% This function has no output args, see OutputFcn.
% hObject    handle to figure
% eventdata  reserved to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% varargin   command line arguments to OSTAnalysis (see VARARGIN)
% Choose default command line output for OSTAnalysis
handles.output = hObject;
% Update handles structure
guidata(hObject, handles);
% UIWAIT makes OSTAnalysis wait for user response (see UIRESUME)
% uiwait(handles.figure1);

% --- Outputs from this function are returned to the command line.
function varargout = OSTAnalysis_OutputFcn(hObject, eventdata, handles)
% varargout cell array for returning output args (see VARARGOUT);
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% Get default command line output from handles structure
varargout{1} = handles.output;
```
% --- Executes on button press in pushbuttonLoad.
function pushbuttonLoad_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonLoad (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

[filename, pathname] = uigetfile({'*.bmp'; '*.tif'; '*.xls'; '*.txt'; '*.*'}, 'Pick a file');
File = [pathname filename];
[path, name, ext, ver] = fileparts(File);
if strcmp(ext, '.bmp') || strcmp(ext, '.BMP') || strcmp(ext, '.tif') || strcmp(ext, '.TIF')
axes(handles.axes1);
imshow(File);
title(['Original OST Image ' filename(1:8)]);
end
 handles.File = File;
 handles.filename = filename;
 handles.pathname = pathname;
guidata(hObject, handles);

% --- Executes on button press in pushbuttonRun.
function pushbuttonRun_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonRun (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

a = find(handles.filename=='0');
a1 = find(a>4);
outFile = [handles.pathname handles.filename(1:a(a1(1))-1) '.xls'];
I = imread(handles.File);
data = double(rgb2gray(I));
[xcor ycor]=getpts;
if get(handles.radiobuttonVideo, 'Value')==0 && count>0 %non-video and more than one image to be processed. The image number must be in order.
frameNum = frameNum0 + count;
if frameNum<10
    handles.filename(b(1)-3:b(1)-1) = ['00' num2str(frameNum)];
elseif frameNum<100
    handles.filename(b(1)-3:b(1)-1) = ['0' num2str(frameNum)];
else
    handles.filename(b(1)-3:b(1)-1) = num2str(frameNum);
end
newfilename = [handles.pathname newfilename(1:1:end-1) '.*.bmp'];
D = dir(fullfile(handles.pathname newfilename));
handles.filename = D.name;
newFile = [handles.pathname handles.filename];
newI = imread(newFile);
data = double(rgb2gray(newI));
axes(handles.axes1);
%imshow(data/255);
imshow(newI);
title(newFile);
[xcor ycor]=getpts;
else %OST video. Frame number must be in order. Use same ROI.
frameNum = frameNum0 + count;
if frameNum<10
    handles.filename(b(1)-3:b(1)-1) = ['00' num2str(frameNum)];
elseif frameNum<100
    handles.filename(b(1)-3:b(1)-1) = ['0' num2str(frameNum)];
else
    handles.filename(b(1)-3:b(1)-1) = num2str(frameNum);
end
else
    handles.filename(b(1)-3:b(1)-1) = num2str(frameNum);
end
newfilename = [handles.filename(1:b(1)-1) '\*' '.bmp'];
D = dir([handles.pathname newfilename]);
handles.filename = D.name;
ewFile = [handles.pathname handles.filename];
newI = imread(newFile);
data = double(rgb2gray(newI));
axes(handles.axes1);
%imshow(data/255);
imshow(newI);
title(newFile);
end
[nline,nsample]=size(data);
xcor(end+1)=xcor(1); ycor(end+1)=ycor(1); % form a close curve
axes(handles.axes2); % select axes 2
% draw a blue color selected area on the image 2 (only for display purpose)
%imshow(data/255);
imshow(newI);
hold on; plot(xcor,ycor,'b','LineWidth',2); hold off
% compute the average temperature in the selected area
bw = poly2mask(xcor,ycor,nline,nsample);
% newimg = data.*bw; % cropped image
% figure, imshow(newimg/255)
[r, c] = find(bw);
selected_no = length(r);
\b = find(handles.filename=='\_');
Lt = str2num(handles.filename(b(1)+1:b(1)+3))/10;
Ht = str2num(handles.filename(b(2)+1:b(2)+3))/10;
cof = (Ht - Lt)/(max(data(:)) - min(data(:)));
data1 = data*cof + Lt;
T1 = data1(ycor(1), xcor(1));
TLN = data1(ycor(2), xcor(2));
TLT = data1(ycor(3), xcor(3));
T4 = data1(ycor(4), xcor(4));
y12 = round((ycor(1)+ycor(2))/2);
x12 = round((xcor(1)+xcor(2))/2);
y34 = round((ycor(3)+ycor(4))/2);
x34 = round((xcor(3)+xcor(4))/2);
T1LN = data1(y12, x12);
T4LT = data1(y34, x34);
for i=1:selected_no
    K(i) = data1(r(i), c(i));
end
Tmin = min(K);
Tmax = max(K);
Tmean = mean(K);
Tsd = std2(K);
GCCx = round((xcor(3) + xcor(2))/2);
GCCy = round((ycor(3) + ycor(2))/2);
Tycc = data1(GCCy, GCCx);
Trtd = (data1(ycor(3), xcor(3)) + data1(ycor(2), xcor(2)))/2 - data1(GCCy, GCCx);
if get(handles.radioButtonLE, 'Value')==0
    Temp = T1;
    T1 = T4;
    T4 = Temp;
    Temp = T1LN;
    T1LN = T4LT;
T4LT = Temp;
Temp = TLN;
TLN = TLT;
TLT = Temp;
end

T0 = [T1 TILN TLN TLT T4LT T4 Tmin Tgcc Tmean Tsd Trtd];
TOS = num2str(T0', '%4.2f');

% T1 = T1*cof+Lt;
% T1N = T1N*cof+Lt;
% TLN = TLN*cof+Lt;
% TLT = TLT*cof+Lt;
% T4T = T4T*cof+Lt;
%
% T4 = T4*cof+Lt;

set(handles.editT1, 'string', T0S(1,:));
set(handles.editT1LN, 'string', T0S(2,:));
set(handles.editLN, 'string', T0S(3,:));
set(handles.editLT, 'string', T0S(4,:));
set(handles.editT4LT, 'string', T0S(5,:));
set(handles.editT4, 'string', T0S(6,:));
set(handles.editMin, 'string', T0S(7,:));
set(handles.editMax, 'string', T0S(8,:));
set(handles.editGcc, 'string', T0S(9,:));
set(handles.editMean, 'string', T0S(10,:));
set(handles.editSd, 'string', T0S(11,:));
set(handles.editRtd, 'string', T0S(12,:));

T10 = xlsread(outFile, 'T1'); %toward nose (left eye)
TILN0 = xlsread(outFile, 'CN'); %TILN (nasal conjunctive)
TLN0 = xlsread(outFile, 'LN'); %nasal limbal
TLT0 = xlsread(outFile, 'LT'); %temporal limbal
T4LT0 = xlsread(outFile, 'CT'); %T4LT (temporal conjunctive)
T40 = xlsread(outFile, 'T4');
Tmin0 = xlsread(outFile, 'Minimum');
Tmax0 = xlsread(outFile, 'Maximum');
Tgcc0 = xlsread(outFile, 'GCC');
Tmean0 = xlsread(outFile, 'MOST'); %Mean
Tsd0 = xlsread(outFile, 'SD');
Trtd0 = xlsread(outFile, 'RTD');

len = length(Tmin0)+1;
T10(len) = T0(1);
TILN0(len) = T0(2);
TLN0(len) = T0(3);
TLT0(len) = T0(4);
T4LT0(len) = T0(5);
T40(len) = T0(6);
Tmin0(len) = T0(7);
Tmax0(len) = T0(8);
Tgcc0(len) = T0(9);
Tmean0(len) = T0(10);
Tsd0(len) = T0(11);
Trtd0(len) = T0(12);

s = size(Tmin0);
if s(1)==1
    Tmin0 = Tmin0';
    Tmax0 = Tmax0';
    Tmean0 = Tmean0';
    Tgcc0 = Tgcc0';
    Tsd0 = Tsd0';
    Trtd0 = Trtd0';
    T10 = T10';
    TILN0 = TILN0';
    TLN0 = TLN0';
    TLT0 = TLT0';
    T4LT0 = T4LT0';
    T40 = T40';
end
xlswrite(outFile, Tmin0, 'Minimum');
xlswrite(outFile, Tmax0, 'Maximum');

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```matlab
% write table row values
xlswrite(outFile, Tgcc0, 'GCC');
xlswrite(outFile, Tmean0, 'MOST');
xlswrite(outFile, Tsd0, 'SD');
xlswrite(outFile, Trtd0, 'RTD');
xlswrite(outFile, T10, 'T1');
xlswrite(outFile, T1LN0, 'CN');
xlswrite(outFile, TLN0, 'LN');
xlswrite(outFile, TLT0, 'LT');
xlswrite(outFile, T4LT0, 'CT');
xlswrite(outFile, T40, 'T4');

set(handles.editFrames, 'string', num2str(FramesNo - count - 1));
end
% executes on button press in radiobuttonVideo.
function radiobuttonVideo_Callback(hObject, eventdata, handles)
% hObject    handle to radiobuttonVideo (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hint: get(hObject,'Value') returns toggle state of radiobuttonVideo

function editMin_Callback(hObject, eventdata, handles)
% hObject    handle to editMin (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of editMin as text
% str2double(get(hObject,'String')) returns contents of editMin as a double

% executes during object creation, after setting all properties.
function editMin_CreateFcn(hObject, eventdata, handles)
% hObject    handle to editMin (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

function editMax_Callback(hObject, eventdata, handles)
% hObject    handle to editMax (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of editMax as text
% str2double(get(hObject,'String')) returns contents of editMax as a double

% executes during object creation, after setting all properties.
function editMax_CreateFcn(hObject, eventdata, handles)
% hObject    handle to editMax (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

function editGcc_Callback(hObject, eventdata, handles)
% hObject    handle to editGcc (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
```

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function editGcc_CreateFcn(hObject, eventdata, handles)
    hObject    handle to editGcc (see GCBO)
    eventdata  reserved - to be defined in a future version of MATLAB
    handles    empty - handles not created until after all CreateFcns called

    % Hint: edit controls usually have a white background on Windows.
    % See ISPC and COMPUTER.
    if ispc && isequal(get(hObject,'BackgroundColor'),
                    get(0,'defaultUicontrolBackgroundColor'))
        set(hObject, 'BackgroundColor', 'white');
    end

function editMean_CreateFcn(hObject, eventdata, handles)
    hObject    handle to editMean (see GCBO)
    eventdata  reserved - to be defined in a future version of MATLAB
    handles    structure with handles and user data (see GUIDATA)

    % Hints: get(hObject,'String') returns contents of editMean as text
    %        str2double(get(hObject,'String')) returns contents of editMean as a double

    % --- Executes during object creation, after setting all properties.
    function editMean_CreateFcn(hObject, eventdata, handles)
        hObject    handle to editMean (see GCBO)
        eventdata  reserved - to be defined in a future version of MATLAB
        handles    empty - handles not created until after all CreateFcns called

        % Hint: edit controls usually have a white background on Windows.
        % See ISPC and COMPUTER.
        if ispc && isequal(get(hObject,'BackgroundColor'),
                        get(0,'defaultUicontrolBackgroundColor'))
            set(hObject, 'BackgroundColor', 'white');
        end

function editSd_CreateFcn(hObject, eventdata, handles)
    hObject    handle to editSd (see GCBO)
    eventdata  reserved - to be defined in a future version of MATLAB
    handles    structure with handles and user data (see GUIDATA)

    % Hints: get(hObject,'String') returns contents of editSd as text
    %        str2double(get(hObject,'String')) returns contents of editSd as a double

    % --- Executes during object creation, after setting all properties.
    function editSd_CreateFcn(hObject, eventdata, handles)
        hObject    handle to editSd (see GCBO)
        eventdata  reserved - to be defined in a future version of MATLAB
        handles    empty - handles not created until after all CreateFcns called

        % Hint: edit controls usually have a white background on Windows.
        % See ISPC and COMPUTER.
        if ispc && isequal(get(hObject,'BackgroundColor'),
                        get(0,'defaultUicontrolBackgroundColor'))
            set(hObject, 'BackgroundColor', 'white');
        end

function editRtd_Callback(hObject, eventdata, handles)
    hObject    handle to editRtd (see GCBO)
    eventdata  reserved - to be defined in a future version of MATLAB
    handles    structure with handles and user data (see GUIDATA)

    % Hints: get(hObject,'String') returns contents of editRtd as text
    %        str2double(get(hObject,'String')) returns contents of editRtd as a double

    % --- Executes during object creation, after setting all properties.
    function editRtd_CreateFcn(hObject, eventdata, handles)
        hObject    handle to editRtd (see GCBO)
function editFrames_Callback(hObject, eventdata, handles)
  % hObject    handle to editFrames (see GCBO)
  % eventdata reserved - to be defined in a future version of MATLAB
  % handles    structure with handles and user data (see GUIDATA)
  % Hints: get(hObject,'String') returns contents of editFrames as text
  % str2double(get(hObject,'String')) returns contents of editFrames as a double

  % --- Executes during object creation, after setting all properties.
  function editFrames_CreateFcn(hObject, eventdata, handles)
    % hObject    handle to editFrames (see GCBO)
    % eventdata reserved - to be defined in a future version of MATLAB
    % handles    empty - handles not created until after all CreateFcns called
    % Hints: edit controls usually have a white background on Windows.
    % See ISPC and COMPUTER.
    if ispc && isequal(get(hObject,'BackgroundColor'),
                    get(0,'defaultUicontrolBackgroundColor'))
        set(hObject,'BackgroundColor','white');
    end

function editLN_Callback(hObject, eventdata, handles)
  % hObject    handle to editLN (see GCBO)
  % eventdata reserved - to be defined in a future version of MATLAB
  % handles    structure with handles and user data (see GUIDATA)
  % Hints: get(hObject,'String') returns contents of editLN as text
  % str2double(get(hObject,'String')) returns contents of editLN as a double

  % --- Executes during object creation, after setting all properties.
  function editLN_CreateFcn(hObject, eventdata, handles)
    % hObject    handle to editLN (see GCBO)
    % eventdata reserved - to be defined in a future version of MATLAB
    % handles    empty - handles not created until after all CreateFcns called
    % Hints: edit controls usually have a white background on Windows.
    % See ISPC and COMPUTER.
    if ispc && isequal(get(hObject,'BackgroundColor'),
                      get(0,'defaultUicontrolBackgroundColor'))
        set(hObject,'BackgroundColor','white');
    end

function editLT_Callback(hObject, eventdata, handles)
  % hObject    handle to editLT (see GCBO)
  % eventdata reserved - to be defined in a future version of MATLAB
  % handles    structure with handles and user data (see GUIDATA)
  % Hints: get(hObject,'String') returns contents of editLT as text
  % str2double(get(hObject,'String')) returns contents of editLT as a double

  % --- Executes during object creation, after setting all properties.
  function editLT_CreateFcn(hObject, eventdata, handles)
    % hObject    handle to editLT (see GCBO)
    % eventdata reserved - to be defined in a future version of MATLAB
    % handles    empty - handles not created until after all CreateFcns called
    % Hints: edit controls usually have a white background on Windows.
    % See ISPC and COMPUTER.
    if ispc && isequal(get(hObject,'BackgroundColor'),
                      get(0,'defaultUicontrolBackgroundColor'))
        set(hObject,'BackgroundColor','white');
    end

function editT1LN_Callback(hObject, eventdata, handles)
  % hObject    handle to editT1LN (see GCBO)
  % eventdata reserved - to be defined in a future version of MATLAB
  % handles    structure with handles and user data (see GUIDATA)
  % Hints: get(hObject,'String') returns contents of editT1LN as text
% str2double(get(hObject,'String')) returns contents of editT1LN as a double

% --- Executes during object creation, after setting all properties.
function editT1LN_CreateFcn(hObject, eventdata, handles)
% hObject    handle to editT1LN (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
    get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

function editT4LT_Callback(hObject, eventdata, handles)
% hObject    handle to editT4LT (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of editT4LT as text
%        str2double(get(hObject,'String')) returns contents of editT4LT as a double

% --- Executes during object creation, after setting all properties.
function editT4LT_CreateFcn(hObject, eventdata, handles)
% hObject    handle to editT4LT (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
    get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

function editT1_Callback(hObject, eventdata, handles)
% hObject    handle to editT1 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of editT1 as text
%        str2double(get(hObject,'String')) returns contents of editT1 as a double

% --- Executes during object creation, after setting all properties.
function editT1_CreateFcn(hObject, eventdata, handles)
% hObject    handle to editT1 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
    get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

function editT4_Callback(hObject, eventdata, handles)
% hObject    handle to editT4 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of editT4 as text
%        str2double(get(hObject,'String')) returns contents of editT4 as a double

% --- Executes during object creation, after setting all properties.
function editT4_CreateFcn(hObject, eventdata, handles)
% hObject    handle to editT4 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
    get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

function editT4_Callback(hObject, eventdata, handles)
% hObject    handle to editT4 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of editT4 as text
%        str2double(get(hObject,'String')) returns contents of editT4 as a double

% --- Executes during object creation, after setting all properties.
function editT4_CreateFcn(hObject, eventdata, handles)
% hObject    handle to editT4 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called
```matlab
% eventdata reserved - to be defined in a future version of MATLAB
% handles   empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
    get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

% --- Executes on button press in pushbuttonCom.
function pushbuttonCom_Callback(hObject, eventdata, handles)
% hObject    handle to pushbuttonCom (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
Num = str2num(get(handles.editFrames,'String'));

b = find(handles.filename=='.');
T1 = 0; T1LN = 0; TLN = 0; TLT = 0; T4LT = 0; T4 = 0;
Tmin = 0; Tmax = 0; Tgcc = 0; Tmean = 0; Tsd = 0; Trtd = 0;
for i=1:Num
    handles.filename(b-1) = num2str(i);
    newFile = [handles.pathname handles.filename];
    
    T1 = T1+xlsread(newFile,'T1');
    T1LN = T1LN+xlsread(newFile,'CN');
    TLN = TLN+xlsread(newFile,'LN');
    TLT = TLT+xlsread(newFile,'LT');
    T4LT = T4LT+xlsread(newFile,'CT');
    T4 = T4+xlsread(newFile,'T4');
    Tmin = Tmin+xlsread(newFile,'Minimum');
    Tmax = Tmax+xlsread(newFile,'Maximum');
    Tgcc = Tmax+xlsread(newFile,'GCC');
    Tmean = Tmean+xlsread(newFile,'MOST');
    Tsd = Tsd+xlsread(newFile,'SD');
    Trtd = Trtd+xlsread(newFile,'RTD');
end
outFile = [handles.pathname handles.filename{1:b-2} '.xls'];

xlswrite(outFile, Tmin/Num, 'Minimum');
xlswrite(outFile, Tmax/Num, 'Maximum');
xlswrite(outFile, Tgcc/Num, 'GCC');
xlswrite(outFile, Tmean/Num, 'MOST');
xlswrite(outFile, Tsd/Num, 'SD');
xlswrite(outFile, Trtd/Num, 'RTD');
xlswrite(outFile, T1/Num, 'T1');
xlswrite(outFile, T1LN/Num, 'CN');
xlswrite(outFile, TLN/Num, 'LN');
xlswrite(outFile, TLT/Num, 'LT');
xlswrite(outFile, T4LT/Num, 'CT');
xlswrite(outFile, T4/Num, 'T4');
```

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APPENDIX 5 – Chapter 5

Appendix 5.1. Summary of results for static measures (Numerical display for Figure 5.2).
Appendix 5.2. Summary of results for dynamic measures (Numerical display for Figure 5.3).
Appendix 5.1. Summary of results for static measures (Numerical display for Figure 5.2).

<table>
<thead>
<tr>
<th></th>
<th>0 s</th>
<th>5 s</th>
<th>10 s</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry eye (n = 62)</td>
<td>Control (n = 63)</td>
<td>p values</td>
<td>Dry eye (n = 62)</td>
</tr>
<tr>
<td>GCC (°C)</td>
<td>34.07 ± 0.52</td>
<td>34.40 ± 0.51</td>
<td>0.0004***</td>
<td>33.65 ± 0.67</td>
</tr>
<tr>
<td>MOST (°C)</td>
<td>34.32 ± 0.51</td>
<td>34.61 ± 0.53</td>
<td>0.0020**</td>
<td>34.05 ± 0.60</td>
</tr>
<tr>
<td>MinT (°C)</td>
<td>33.60 ± 0.53</td>
<td>33.89 ± 0.66</td>
<td>0.0077**</td>
<td>33.16 ± 0.75</td>
</tr>
<tr>
<td>MaxT (°C)</td>
<td>35.33 ± 0.49</td>
<td>35.60 ± 0.54</td>
<td>0.0050**</td>
<td>35.23 ± 0.51</td>
</tr>
<tr>
<td>T1 (°C)</td>
<td>35.11 ± 0.53</td>
<td>35.29 ± 0.89</td>
<td>0.1725</td>
<td>35.01 ± 0.55</td>
</tr>
<tr>
<td>T4 (°C)</td>
<td>34.31 ± 0.54</td>
<td>34.66 ± 0.56</td>
<td>0.0005***</td>
<td>34.21 ± 0.58</td>
</tr>
<tr>
<td>CT (°C)</td>
<td>34.53 ± 0.51</td>
<td>34.81 ± 0.61</td>
<td>0.0070**</td>
<td>34.33 ± 0.58</td>
</tr>
<tr>
<td>LT (°C)</td>
<td>34.13 ± 0.51</td>
<td>34.43 ± 0.51</td>
<td>0.0013**</td>
<td>33.81 ± 0.59</td>
</tr>
<tr>
<td>LN (°C)</td>
<td>34.61 ± 0.46</td>
<td>34.80 ± 0.64</td>
<td>0.0591</td>
<td>34.31 ± 0.55</td>
</tr>
<tr>
<td>CN (°C)</td>
<td>35.06 ± 0.45</td>
<td>35.25 ± 0.84</td>
<td>0.1296</td>
<td>34.88 ± 0.50</td>
</tr>
</tbody>
</table>

Data are the mean ± SD; * p < 0.05; ** p < 0.01; *** p < 0.001
Appendix 5.2. Summary of results for dynamic measures (Numerical display for Figure 5.3).
0s

1s

2s

3s

4s

5s

6s

7s

8s

9s

10s

0
0

GCC (°C)

D
C
p

-0.18±0.20
-0.17±0.16
0.813

-0.26±0.21
-0.24±0.23
0.727

-0.31±0.23
-0.28±0.26
0.576

-0.36±0.27
-0.33±0.30
0.618

-0.41±0.31
-0.37±0.33
0.429

-0.46±0.35
-0.40±0.34
0.304

-0.49±0.37
-0.43±0.39
0.450

-0.52±0.37
-0.48±0.40
0.579

-0.55±0.40
-0.53±0.42
0.735

-0.60±0.43
-0.55±0.45
0.520

0
0

MOST (°C)

D
C
p

-0.08±0.07
-0.09±0.09
0.413

-0.14±0.10
-0.14±0.13
0.956

-0.19±0.12
-0.18±0.16
0.899

-0.22±0.14
-0.21±0.18
0.602

-0.27±0.17
-0.24±0.21
0.401

-0.29±0.19
-0.26±0.21
0.289

-0.31±0.21
-0.28±0.24
0.366

-0.33±0.21
-0.31±0.25
0.49

-0.35±0.23
-0.32±0.26
0.487

-0.38±0.25
-0.35±0.28
0.517

0
0

MnT (°C)

D
C
p

-0.18±0.18
-0.18±0.18
0.832

-0.27±0.24
-0.26±0.25
0.883

-0.34±0.29
-0.31±0.30
0.739

-0.39±0.32
-0.36±0.33
0.725

-0.45±0.37
-0.40±0.35
0.542

-0.50±0.42
-0.44±0.38
0.416

-0.52±0.43
-0.49±0.41
0.675

-0.55±0.47
-0.51±0.42
0.707

-0.57±0.47
-0.55±0.43
0.897

-0.61±0.49
-0.57±0.45
0.773

0
0

MaxT (°C)

D
C
p

-0.03±0.06
-0.02±0.06
0.665

-0.05±0.09
-0.03±0.09
0.471

-0.07±0.09
-0.05±0.11
0.251

-0.08±0.11
-0.05±0.13
0.086

-0.11±0.13
-0.05±0.16
0.037*

-0.13±0.13
-0.06±0.16
0.012*

-0.13±0.13
-0.06±0.18
0.022*

-0.14±0.13
-0.07±0.19
0.023*

-0.16±0.15
-0.08±0.19
0.016*

-0.17±0.17
-0.09±0.21
0.019*

0
0

T1 (°C)

D
C
p

-0.02±0.07
-0.03±0.07
0.487

-0.04±0.11
-0.04±0.08
0.919

-0.05±0.12
-0.06±0.09
0.958

-0.07±0.14
-0.05±0.11
0.393

-0.10±0.15
-0.07±0.12
0.214

-0.11±0.15
-0.06±0.13
0.053

-0.13±0.17
-0.07±0.13
0.057

-0.15±0.16
-0.09±0.15
0.052

-0.17±0.19
-0.10±0.15
0.053

-0.18±0.22
-0.12±0.16
0.103

0
0

T4 (°C)

D
C
p

0.01±0.16
-0.02±0.10
0.225

-0.04±0.21
-0.02±0.13
0.47

-0.07±0.23
0.01±0.14
0.023*

-0.09±0.22
0.01±0.13
0.005**

-0.10±0.24
0.00±0.19
0.014*

-0.08±0.24
0.01±0.16
0.009**

-0.07±0.26
0.01±0.19
0.047*

-0.08±0.27
0.02±0.23
0.036*

-0.07±0.23
0.02±0.25
0.028*

-0.09±0.22
0.04±0.24
0.005**

0
0

CT (°C)

D
C
p

-0.05±0.11
-0.07±0.08
0.206

-0.10±0.14
-0.12±0.11
0.581

-0.13±0.16
-0.15±0.15
0.554

-0.18±0.19
-0.16±0.18
0.522

-0.20±0.21
-0.18±0.20
0.569

-0.22±0.22
-0.20±0.20
0.5

-0.22±0.24
-0.21±0.21
0.88

-0.26±0.25
-0.23±0.21
0.424

-0.27±0.26
-0.24±0.22
0.463

-0.29±0.29
-0.25±0.23
0.397

0
0

LT (°C)

D
C
p

-0.11±0.15
-0.13±0.15
0.474

-0.17±0.17
-0.22±0.21
0.192

-0.22±0.20
-0.28±0.27
0.163

-0.28±0.23
-0.31±0.29
0.504

-0.32±0.24
-0.35±0.32
0.574

-0.35±0.26
-0.36±0.34
0.888

-0.36±0.29
-0.40±0.37
0.495

-0.41±0.30
-0.44±0.39
0.65

-0.43±0.32
-0.46±0.40
0.65

-0.46±0.34
-0.49±0.42
0.709

0
0

LN (°C)

D
C
p

-0.08±0.14
-0.10±0.12
0.519

-0.16±0.17
-0.17±0.16
0.945

-0.22±0.20
-0.20±0.18
0.557

-0.25±0.21
-0.23±0.22
0.494

-0.30±0.24
-0.26±0.25
0.353

-0.34±0.27
-0.28±0.27
0.252

-0.36±0.28
-0.30±0.30
0.276

-0.39±0.29
-0.35±0.33
0.506

-0.40±0.32
-0.37±0.35
0.63

-0.45±0.34
-0.40±0.38
0.525

D 0
-0.06±0.07
-0.09±0.10
-0.12±0.12
C 0
-0.06±0.09
-0.09±0.12
-0.11±0.14
p
0.909
0.767
0.794
Data are the mean ± SD; D: Dry eye; C: Control; * p < 0.05; ** p < 0.01

-0.15±0.13
-0.13±0.17
0.411

-0.18±0.15
-0.15±0.18
0.276

-0.19±0.17
-0.16±0.19
0.291

-0.21±0.18
-0.18±0.20
0.318

-0.23±0.18
-0.19±0.22
0.291

-0.25±0.20
-0.21±0.22
0.337

-0.26±0.21
-0.22±0.23
0.295

CN (°C)

254

