Title
The Use of Infrared Thermography and the Pain Sensitivity Questionnaire to Understand Evaporative Dry Eye

Author
Li, Wing Y

Publication Date
2016

Peer reviewed|Thesis/dissertation

The Use of Infrared Thermography and the Pain Sensitivity Questionnaire to Understand Evaporative Dry Eye

By

Wing Y. Li

A dissertation submitted in partial satisfaction of the
requirement for the degree of
Doctor of Philosophy

in

Vision Science

in the

Graduate Division

of the

University of California, Berkeley

Committee in Charge:

Professor Meng C. Lin, Chair
Professor Steve Selvin
Professor Clayton Radke

Spring 2016

Abstract

The Use of Infrared Thermography and the Pain Sensitivity Questionnaire to Understand Evaporative Dry Eye

By

Wing Y Li

Doctor of Philosophy in Vision Science

University of California, Berkeley

Professor Meng C. Lin, Chair

Dry eye is marked by symptoms of ocular irritation, fatigue, photophobia and other symptoms resulting from ocular surface damage.[1] The most prevalent form of dry eye is evaporative dry eye (EDE), which is primarily due to a decreased quality and quantity of the tear lipid layer. The tear lipid layer is an important component to the tear film, acting as a barrier against tear evaporation, inhibiting it by approximately 75-90%. Excessive tear-film evaporation leads to increased tear salinity, resulting in long-term phenotypic and pro-inflammatory changes to the cornea. Unfortunately, there has only been limited development in treatment options for EDE, due to low repeatability of clinical test and poor correlation with symptoms seen in current diagnostic tests.

This has served as an impetus to look for new ways to diagnose it, with recent research focused on using infrared thermography (IRT) to assess ocular surface cooling (OSC) as an indirect measure of tear film evaporation. This is based on the theory that when tear evaporation occurs, the phase change from liquid to gas is associated with heat transfer to the surrounding environment, and thus OSC. This was not conclusively demonstrated until our study; more importantly, the study showed that localized areas of OSC represented regions of tear lipid layer breakup, which was important as it is thought to be where the discomfort associated with EDE originates.

Once a greater understanding of what OSC represented was gained, it was important to define the repeatability of it. For the measurement to be clinically significant, a study was designed that determined the inter-day and intra-day profile repeatability, which was found to have a good inter-day repeatability in an EDE cohort. Next, it was determined that IRT appeared to overcame a major issue found with current EDE tests, which is the poor association between signs and symptoms.

Even if tear-film instability reflects the level of irritation on the ocular surface, it is important to recognize that ocular discomfort experienced by an individual is not solely defined by physical disruption to the ocular surface, but by how cognition influences its perception. One of the most important cognitive factors in influencing pain perception is pain sensitivity, as it is linked with analgesic use after surgery and risk of developing chronic pain. Research on this topic has been limited, as measuring pain sensitivity involves determining the level of induced pain that can be tolerated, making it time-intensive, expensive and requires inducing pain in healthy subjects. The Pain Sensitivity

Questionnaire (PSQ) remedies this issue, as it is a self-rating instrument that asks individuals to rate the pain they feel they would experience in imagined painful situations. In our work, a higher PSQ score (i.e., greater pain sensitivity) was associated with increased dry eye symptoms and contact lens discomfort.

In conclusion, the work described in the dissertation appears to suggest the viability of a novel diagnostic test for dry eye; IRT is objective, repeatability and seems to be associated with ocular discomfort. Nevertheless, IRT cannot solely be used to understand the connection between signs and symptoms of dry eye but will rely on also recognizing that the inter-individual variability in pain sensitivity plays a significant role in defining that relationship. Significant advances in EDE were made but further understanding of how tear-film instability induces ocular discomfort is needed.

Dedication

To the loving memory of my father, John Li (February 13, 1949-February 9, 2010) and my mother, Chui Ho Kan (March 29, 1951- March 23, 2004) for all the hard work and sacrifice to raise me and my brother.

Acknowledgements

I would like to first acknowledge the mentorship provided by Dr. Meng C. Lin. She deserves recognition for all the time and effort she has spent on mentoring students during her career. This dissertation would be impossible without Dr. Lin's patience, encouragement, support and expert knowledge, for which I am indebted to her. Her influence has not only made me into a better clinician and researcher but also most importantly into a better person.

I would like to thank the rest of my committee members, Dr. Clayton Radke and Dr. Steve Selvin for their insights, expertise and encouragement to think outside the box.

A special thanks goes out to Dr. Richard Lubman, Dr. Alan S. Yu and Dr. Suzanne Fleiszig for each giving me the opportunity to work in their respective labs. The years spent reading papers, attending lab meetings, and working on projects gave me the confidence in knowing that I could be a scientist. I also appreciate all the career advice that Dr. Fleiszig has offered.

The studies described in this dissertation would not have been possible without the hard work and support of the Clinical Research Center (CRC) staff. I would like to thank (in alphabetical order) Annie Lee, Bo Tan, Carly Childs, Kathy Le, Kristina Lin, Lurline Liu, Mariel Lerma, Michelle Wong, Miguel Agreda, Dr. Nina Tran, Salena Thong, Dr. Thao Yeh, Dr. Tiana Leung, Dr. Tiffany Yuen and Tom Dursch. I would also like to directly acknowledge two staff members: Andrew (Andy) Graham and Uyen Do. I would like to thank Andy for teaching me how to be a better writer and training me to be a biostatistician, and to Uyen for being like a sister to me during my time at the CRC; words cannot describe how much I have cherished her friendship over these years.

I would like to thank my family and friends, who without their emotional and spiritual support, I could never have made it through my PhD program. To my wonderful brother, Alan Li, and sister-in-law, Kalam Lok, for unequivocally supporting me throughout the entire process. To my awesome girlfriend, Cheryl Wong, for encouraging me and being there for me during my moments of self-doubt. To my dear friends and members of my church community, for offering their love and support.

Last but not least, I would like to thank Jesus, my lord and savior, for his blessings and guidance throughout my life.

Wing (Eric) Li, OD, PhD, FAAO

University of California, Berkeley
School of Optometry
110B Minor Hall, Berkeley, CA, 94720

Telephone: 626-863-5132
E-mail: ericli13@berkeley.edu

Education

University of California Berkeley 2012-2016
- PhD in Vision Science
- GPA: 3.67/4.00
- Research Areas: Meibomian Gland Dysfunction, Pain Sensitivity, Ocular Surface Temperature, Computer Vision Syndrome

University of California Berkeley School of Optometry 2010-2011
- Optometry Residency
- Specialty: Ocular Disease and Contact Lens

University of California Berkeley School of Optometry 2006-2010
- OD, Doctor of Optometry
- GPA: 3.81/4.00

University of California Berkeley 2002-2006
- B.A. Molecular Cell Biology
- GPA: 3.39/4.00

Clinical and Work Experience

Consultant, EyePACS: Telemedicine Retinal Screening Program 2011-Current
Supervisor: Jorge Cuadros, OD, PhD

Optometric Associate, Eye-Care Optometry 2011-Current
Supervisor: Ursula Moonsamy, OD

Clinical Mentor, California Glaucoma Certification Course (BOLD) 2011-Current
Supervisor: Carl Jacobsen, OD

Clinical Instructor, University of California Berkeley School of Optometry 2011-2012
Supervisor: Edward Revelli, OD

Research Optometrist, Clinical Research Center, University of California Berkeley School of Optometry 2011-2012
Supervisor: Meng C. Lin, OD, PhD, FAAO

Research Assistant, University of California Berkeley 2006-2009
Supervisor: Suzanne Fleiszig, OD, PhD, FAAO

Lab Technician, University of California Berkeley 2005-2009
Supervisor: Patricia Zambryski, PhD

Research Assistant, University of Southern California Medical School 2003-2005
Supervisor: Alan Yu, MD

Research Assistant, University of Southern California Medical School 2000-2002
Supervisor: Richard Lubman, MD

Teaching and Mentoring Experience

Research Supervisor, University of California Berkeley, Berkeley California

- Diana Yan, Mariel Lerma, Kathy Le, Kristina Lin

Teaching Assistant, University of California Berkeley School of Optometry, Berkeley, California

- In "Vision Science 203A: Geometrical Optics" Aug 2012- Dec 2012
  Aug 2013 - Dec 2013

Clinical Instructor, University of California Berkeley School of Optometry, Berkeley, California

- In "Vision Science 440A-C: Advanced Optometry Clinic" Jul 2011- Jul 2012
- Instructed 3rd and 4th year Optometry Students
- Instructed Optometry Residents

Awards and Fellowships

1. Michael G. Harris Ezell Fellowship (2014)

2. Michael G. Harris Ezell Fellowship (2013)

3. George L. Schneider Memorial Scholarship (2009)

Peer-Reviewed Publications

1. **Li W**, Graham AD, Lin MC. Understanding ocular discomfort and dryness using the Pain Sensitivity Questionnaire. PLOS ONE. In Press.

2. **Li W**, Graham AD, Lin MC. Ocular Surface Cooling Corresponds to Tear Film Thinning and Breakup. Optometry and Vision Science. 2015 Sep;92(9):e248-56.

3. Pucker AD, Jones-Jordan LA, Srinivasan S, **Li W**, Kwan JT, Lin MC, Sickenberger W, Jones L. Associations with Meibomian Gland Atrophy in Daily Contact Lens Wearers. Optometry and Vision Science. 2015 Sep;92(9):e206-13.

4. **Li WY**, Hsaio C, Graham AD, Lin MC. Corneal Epithelial Permeability: Ethnic Differences between Asians and non-Asians. Contact Lens and Anterior Eye. 2013 Oct;36(5):215-8.

5. Borkar DS, Fleiszig SM, Leong C, Lalitha P, Srinivasan M, Ghanekar AA, Tam C, **Li WY**, Zegans ME, McLeod SD, Lietman TM, Arharya NR. Association between Cytotoxic and Invasive Pseudomonas aeruginosa and Clinical Outcomes in Bacterial Keratitis. Archives of Ophthalmology, 2013 Feb 1;131(2):147-153

6. Augustin DK, Heimer SR, Tam C, **Li WY**, Le Due JM, Evans JM, Fleiszig SM. Role of defensins in corneal epithelial barrier function against Pseudomonas aeruginosa transversal. Infection and Immunity. 2011 Feb; 79(2):595-605.

7. Tam C, Lewis SE, **Li WY**, Lee E, Evans DJ, Fleiszig SM. Mutation of the phospholipase catalytic domain of the Pseudomonas aeurginosa cytotoxin ExoU abolishes colonization promoting activity and reduces corneal disease severity. Experimental Eye Research. 2007 Dec; 85(6):799-805.

8. **Li WY**, Huey CL, Yu AS. Expression of Claudin 7 and 8 along the mouse nephron. American Journal Physiology: Renal Physiology 286: F1063-F1070, 2004.

Presentations

1. **Li WY**, Graham AD, Lin MC. Repeatability of Ocular Surface Cooling Measurement. Association for Research in Vision and Ophthalmology Meeting 2016. Abstract #2850 – A0059.

2. Radke CJ, Dursch TJ, **Li W**, Taraz B, Lin MC. Human Tear-Film Evaporation Rates from Infrared Ocular-Surface Cooling and Fluorescein Breakup. Association for Research in Vision and Ophthalmology Meeting 2016. Abstract #6168 – A0071.

3. Lin MC, Graham AD, Satjawatcharaphong P, **Li WY**, Yeh T, Lerma M, Lin K. Tear Lipid Layer Thickness and Variability Both Impact Tear Film Stability. Association for Research in Vision and Ophthalmology Meeting 2016. Abstract #3442.

4. **Li WY**, Graham AD, Lin MC. Repeated Tear Film Stress Associated with Increased Rate of Ocular Surface Cooling. American Academy of Optometry 2015: Dry Eyes

Pathophysiology and Treatment Super Session.

5. Lin MC, **Li WY**, Radke CJ. Localized Cooling Precedes Tear-Film Breakup. International Society for Contact Lens Research Symposium 2015.

6. Jones-Jordan LA, Pucker AD, **Li W**, Kwan JT, Lin MC, Sickenberger W, Jones LW. A Comparison of Meibomian Gland Expressibility Methods in Contact Lens and Non-Contact Lens Wearers. British Contact Lens Association Clinical Conference 2015, Poster Board Dry-41.

7. **Li WY**, Graham AD, Lin MC. Ocular Surface Cooling as a Potential Stimulus for Blinking. Association for Research in Vision and Ophthalmology Meeting 2015, Poster board #D0250.

8. **Li W**, Graham AD, Lin MC. Understanding Ocular Discomfort and Dryness using the Pain Sensitivity Questionnaire. American Academy of Optometry 2014: Hot Topics Dry Eye Session.

9. Yuen T, Kitamata-Wong B, Zhou Y, **Li W**, Lin MC. Effects of multi-purpose lens care solutions on comfort and tear film stability. American Academy of Optometry 2014.

10. Kitamata-Wong B, Yuen T, Zhou Y, **Li W**, Lin MC. Effects of lens-care solutions on corneal epithelial integrity. American Academy of Optometry 2014.

11. Srinivasan S, Pucker AD, Jones-Jordan LA, **Li W**, Kwan JT, Sickenberger W, Marx S, Lin MC, Jones L. Meibomian Gland Atrophy Rate in Contact Lens and Non-Contact Lens Wearers. American Academy of Optometry 2014.

12. Pucker AD, Jones-Jordan LA, Srinivasan S, **Li W**, Kwan JT, Lin MC, Sickenberger W, Jones L. Factors Associated with Meibomian Gland Atrophy in Daily Contact Lens Wearers. American Academy of Optometry 2014.

13. **Li WY**, Graham AD, Lin MC. Ocular Surface Cooling Corresponds to Tear Film Thinning and Breakup. Association for Research in Vision and Ophthalmology Meeting 2014. Poster board #B0299.

14. Niimi J, Tan B, Chang J, Zhou Y, Ghanekar A, **Li W**, Lee A, Wong M, Lin MC. Diurnal Variation of Tear Osmolarity and Its Relationship with Central Corneal Thickness Over a 14-hr Period. Association for Research in Vision and Ophthalmology Meeting 2012.

15. Tan B, Graham AD, Zhou Y, Ghanekar A, Niimi J, **Li W**, Lin MC. A Novel Analytical Method to Quantitatively Describe the Corneoscleral Junction Using Optical Coherence Tomography (OCT). Association for Research in Vision and Ophthalmology Meeting 2012.

16. Tam C, Lewis SE, **Li WY**, Lee E, Evans DJ, Fleiszig SM. Mutation of the phospholipase catalytic domain of the Pseudomonas aeurigonisa cytotoxin ExoU abolishes colonization promoting activity and reduces corneal disease. Association for Research in Vision and Ophthalmology Meeting 2007.

Research Support

T32EY007043
Lin (PI); Li (Trainee) 08/01/2012 – 07/31/2015
Title: Understanding evaporative dry eye symptoms using infrared thermography and the pain sensitivity questionnaire.
The goal of this study is to use infrared thermography and the pain sensitivity questionnaire to understand the symptoms associated with evaporative dry eye.
Role: Post-doctoral Trainee

T35EY007139-15
Fleiszig (PI); Li (Trainee) 06/01/2008 – 08/31/2008
Title: Association between cytotoxic and invasive *Pseudomonas aeruginosa* and clinical outcomes in bacterial keratitis.
The goal of this study was to determine whether cytotoxic and invasive *Pseudomonas aeurginosa* had different clinical presentations and outcomes to a topical moxifloxacin and corticosteroid treatment.
Role: Pre-doctoral Trainee

T35EY007139-14
Fleiszig (PI); Li (Trainee) 06/01/2007 – 08/31/2007
Title: Mutation of the phospholipase catalytic domain of the *Pseudomonas aeurginosa* cytotoxin ExoU abolishes colonization promoting activity and reduces corneal disease severity.
The goal of this study was to determine whether *Pseudomonas aeurginosa* used phospholipase activity to mediate
Role: Pre-doctoral Trainee

Table of Contents

List of Figures

List of Tables

List of Abbreviations

^{0}C	Degree Celsius
^{0}C/sec	Degree Celsius per Second
AM	Morning
CL	Contact Lens
CLW	Contact Lens Wearers
CVS	Computer Vision Syndrome
DE	Dry Eye
DEFC	Dry Eye Flow Chart
EDE	Evaporative Dry eye
EOD	End of Day
FDA	Federal Food and Drug Administration
FTBU	Fluorescein Tear Breakup
ICC	Intraclass Correlation Coefficient
IED-C	Inter-Eye Difference in Comfort
IED-D	Inter-Eye Difference in Dryness
IRT	Infrared Thermography
LoA	Limits of Agreement
MIBP	Maximum Interblink Period
MGD	Meibomian Gland Dysfunction
mm	Millimeter
NITBUT	Non-invasive Tear Breakup Time
OSC	Ocular Surface Cooling

OSDI	Ocular Surface Disease Index
OST	Ocular Surface Temperature
PM	Afternoon
PSQ	Pain Sensitivity Questionnaire
PSQ-min	Pain Sensitivity Questionnaire Minor Subscore
PSQ-mod	Pain Sensitivity Questionnaire Moderate Subscore
SD	Standard Deviation
Sec	Second
TC	Thermographic Camera
TER	Tear Evaporimeter
TFLL	Tear Film Lipid Layer
TRPM-8	Transient Receptor Potential Cation Channel, subfamily M, member 8
VAS	100-point Visual Analog Scale
V1	Visit 1
V2	Visit 2

Chapter 1: General Introduction

Dry eye is one of the common ocular morbidities in the world, affecting nearly 5-30% of the world population.[1–5] Sufferers often report symptoms of ocular irritation, fatigue, photophobia, itchiness, excessive tearing, redness and a host of other symptoms, which is attributed to issues with the tear film.[1–5] The tear film is a 3-5 micron thick layer of fluid that provides lubrication, nutrients and moisture necessary for clear vision and protection of the ocular surface (Figure 1-1).[6–8] Severe dry eye can lead to permanent sight loss but even mild to moderate dry eye is associated with a decreased quality of life and increased risk for depression.[9–14]

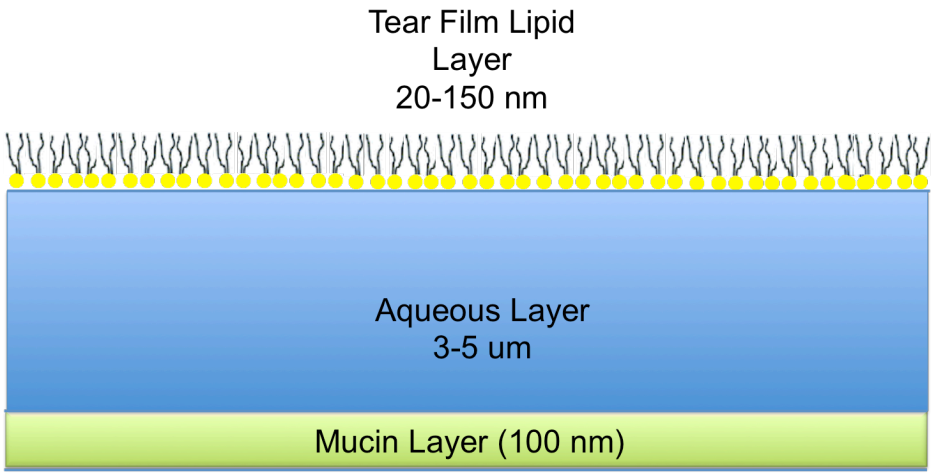

*Figure not drawn to scale

Figure 1-1 Schematic drawing of the tear film, with the air-tear film interface at the top of the image.

Sufferers are often forced to make significant life adjustments such as discontinuing contact lens wear, using artificial tears five to ten times a day, having plastic/collagen plugs inserted in their punctum, changing professions to minimize computer use or using humidity swim goggles to function. Risk factors for dry eye include age, being female, taking certain medications (e.g. anti-depressants and hypertensive medications), in dry or windy environmental conditions, and having systemic conditions (e.g. diabetes and arthritis).[1–5] The annual economic cost to diagnose and treat dry eye in the United States is estimated to be 55.4 billion dollars, which does not factor that sufferers often experience a significant decline in worker productivity.[15–17] Even with the significant social and economic cost associated with dry eye, there are few treatments available as many aspects of dry eye are still not well understood. Recent progress has been made with the recognition that dry eye is a multi-factorial condition.[18]

The International Dry Eye Workshop in 2007 identified evaporative dry eye (EDE) as the most prevalent type of dry eye, with meibomian gland dysfunction (MGD) as the

primary cause.[19] The meibomian glands are sebaceous glands, located along the upper and lower eyelid margins, which secrete a mixture of various lipids and proteins, known as meibum, on the ocular surface.[20] MGD can lead to the alteration of the tear film lipid layer (TFLL), causing less meibum to be secreted due to meibomian gland dropout or obstruction to the glands. This is an issue because the TFLL is a 50-100 nm layer of lipid, proteins and other constituents that play a major role in inhibiting tear evaporation due to its high local mass transfer resistance, which is theorized to decrease tear evaporation by 75-90%.[21–23]

Lacking the TFLL would lead to excessive aqueous tear-film evaporation rates, which contributes to tear hyperosmolarity, leading to ocular discomfort. Mathematical models and *in vitro* studies suggest that the TFLL does not lose its evaporation inhibitory properties because it is thinner but as a result of localized areas of TFLL rupture (i.e., absence of TFLL) that form during an inter-blink period.[24,25] Without the TFLL, an area of the ocular surface would experience elevated tear evaporation, leading to tear hyperosmolarity and ocular discomfort.[25–28] More importantly, there are likely long-term phenotypic changes that potentially contribute to neuropathic pain and disruption of the corneal-lacrimal feedback loop.[20,26,29–33] Increased tear evaporation may also be an important contributor to contact lens discomfort and likely the most prevalent cause for contact lens wear discontinuation, which affects nearly 50% of all lens wearers.[34,35]

A decreased blink rate may also play an important role in causing EDE as blinking is required for secretion and spread of meibum over the ocular surface.[36–38] This is important due to the increased computer use in everyday life, with its use in the workplace doubling in the last twenty-five years.[39] In addition, it is no longer limited to the workplace but has increasingly encompassed all aspects of life, with significant time spent on social media, entertainment and communication. As a result, Americans spend an average of 8.5 hours a day in front of a digital device (e.g., computer, smartphone).[40] An issue due to the tear film change that manifest after individuals spend an extended period on a computer; this is likely attributed to the 50-60% reduction in blink rate when common computer tasks are done (e.g., writing an e-mail or reading the news), due to an increased cognitive load.[41–43] Although blink rate is similarly reduced when reading a book or listening to music, the computer's multi-functionality (e.g., web browsing, working on word documents or spreadsheets, watching video clips, etc.) allows individuals to spend countless hours on it while only taking minimal breaks.[44] The ubiquitous use of computers and other digital device may lead to a drastic increase in the prevalence of EDE, creating a modern widespread condition.

Even with the significant socioeconomic burden caused by EDE, only limited development in treatment options has been made. Restasis®, which was approved in 2003, is currently the only FDA-approved medication for dry eye, which increases tear production but likely has minimal effect on EDE, as it does not help with the underlying pathophysiology of the condition. The lack of new treatment options in over 10 years is partially due to the lack of an adequate diagnostic test for EDE.[45] Fifteen companies (including Alcon, Benebiosis, Incyte, Rigel, Lux, Allostera, Gtx, Ligand, Acadia, Aciex, AmorePacific, etc.) have sought and failed to get FDA approval for their dry eye drugs. Several drugs, most recently Eleven Biotherapeutics' EBI-005 drug, were unable to pass Phase 3 clinical trials due to the inability to show an improvement in signs *and*

symptoms. Even Shire's Liftegrast, which experts have identified as the medication most likely to obtain FDA approval, has had to run three separate Phase 3 randomized clinical trials with over 2,500 subjects at great expense, and may be required to run a fourth trial before it is approved. Meeting both requirements for signs and symptoms was not compulsory when Restasis® was FDA approved, but a major impediment in developing novel treatments for EDE is the lack of an adequate diagnostic test, which has limited potential for new treatment options.[45,46] Current diagnostic tests are compromised by their poor accuracy, low repeatability and most importantly, lack of association between signs and symptoms.[18,45,47,48]

Clinicians are faced with a lack of clinical test that can accurately and objectively diagnose and monitor EDE.[18,47,48] Mathematical models have been done to provide theories to explain the pathophysiology of EDE by MGD, but there has been a general lack of clinical work done to confirm the models, due to limitations in the tools available to clinically assess tear evaporation.[23,25] Tear evaporimeters (TERs), which uses humidity sensors to determine the difference in relative humidity between an open and closed eye, has been the method most commonly employed to measure tear evaporation rate.[6,49,50] Though TERs have been used to elucidate features of EDE, limitations of TERs likely hinder its effectiveness in EDE research.[6,49,50] The first issue is that most TERs can only measure tear evaporation rates in a closed environment, which underestimates tear evaporation rates, as restriction of air movement on the tear film inhibits evaporation.[51] In addition, tear evaporation rates provided by TERs represent an aggregate measure over several blink; the slow response reflects the time needed for water molecules to reach environmental equilibrium. The high variability of the tear film, even between blinks, suggests that a more dynamic measure of tear evaporation rates would be optimal.[28] Finally, TERs provides tear evaporation rates over the entire ocular surface but the localized region of increased evaporation are likely key in causing EDE symptoms.[25,26]

The limitations of the TERs have prompted recent interest in infrared thermography (IRT) to measure ocular surface temperature (OST) as a method to indirectly assess tear evaporation rates. Mapstone first adapted the IRT to measure the temperature of the ocular surface, and other researchers later implemented the technique to investigate the role of OST in DE.[52,53] Recent research has focused on using the IRT to measure tear film evaporation indirectly, based on the theory that when tear evaporation occurs, the phase change from liquid to gas is associated with heat transfer to the surrounding environment, and thus a cooling of the ocular surface.[54–59] It is known that liquids with a higher rate of evaporation evince a greater rate of surface cooling; as an example, when ethanol and water are applied to the skin, the area treated with ethanol will decrease in surface temperature more rapidly than the area with water applied, because the rate of evaporation is higher for ethanol.[60,61] It is reasonable to hypothesize that tear evaporation leading to tear film thinning and breakup should be associated with concomitant ocular surface cooling (OSC).[28,54,55,62]

These relationships, however, were not conclusively demonstrated, as some studies reported an association between OST and tear film stability, while others have found no such association.[56,58,63–66] A possible reason for the conflicting results is that most studies measured OST and tear film stability separately – a significant issue given

the highly variable and dynamic nature of the tear film.[54,56,57,63,67–69] This uncertainty served as the impetus for our first study, which is described in Chapter 2 of the thesis.

Once we obtained a more detailed understanding of what IRT measures in terms of the tear-film, we identified that it there has only been limited work to define the repeatability of OST and OSC rate; without this information it is difficult to provide significant insight on the clinical potential of OST measurements. For it to be clinically useful in the diagnosis and monitoring of EDE, a profile on inter-day and intra-day repeatability must be determine, which was the purpose the study described in Chapter 3 of the thesis.

After this study, we felt that IRT demonstrated characteristics that potentially made it a possible gold standard test for EDE as it was repeatable, objective, and provides an easily interpretable metric, the OSC rate, which could be used to monitor and educate patients with EDE. The IRT may overcome one of the major issues with current EDE tests, which is the poor association between signs and symptoms.[29,46,70] Animal *in vivo* models have identified transient receptor potential cation channel, subfamily M, member 8 (TRPM-8) as the class of nociceptors likely responsible for symptoms experienced during EDE.[33,71–74] Animal *in vivo* models measure nerve activation using extracellular single-unit recording of single neurons in the trigeminal ganglion, and determined that TRPM-8 are activated by hyperosmolarity or cooling; significantly, a synergistic activation is observed when both stimuli are applied.[33,75] With evidence to suggest that a localized region of elevated tear evaporation leads to cooling and hyperosmolarity, we hypothesized that IRT could be used to quantify the level of ocular surface irritation in EDE, with a higher OSC rate associated with greater discomfort and that IRT may be used to quantify the irritation (i.e. tear film stress) on the ocular surface.[25,26,32,76] This hypothesis led us to conduct the study described in Chapter 4 of the thesis. The study also provided additional insight into the important role that blinks play on the formation and maintenance of the TFLL during an inter-blink period.

Though IRT may provide a method to assess the tear evaporation rate, it may be important to recognize that the ocular discomfort experienced by an individual is not solely defined by the physical disruption of the ocular surface but also on the neurological factors that affects how it is perceived.[29,77–79] Even if IRT measures ocular surface irritation, it may not translate to understanding how it is ultimately perceived, as pain sensitivity is highly individualized.[80–82] It would not be unexpected if an identical ocular irritant, applied to a group of individuals, led to a diverse range in the level of discomfort reported.[80,83,84] It is a similar issue that faced pain researchers nearly 20 years ago.[80,85–87]

Until recently, pain research was primarily focused on the cause and treatment of pain but it had difficulty explaining why patients reported a wide range of pain from the same injury.[80,85,86] It was only with the recognition that the cognitive modulation of pain is highly individualized, which has provided an insight into why individuals experience pain so differently.[80,85,86] Modern pain researchers interpreted this using the biopsychosocial pain model, which as Green eloquently states “that pain is ultimately sculpted by complex and dynamic interactions among biological, psychological and sociocultural processes.”[88] Recent studies have provided support for this model as pain perception has been found to be influenced by factors such as ethnicity, culture, anxiety and depression.[82,89–94]

The neural pathway for ocular discomfort reveals how the biopsychosocial pain model may influence the perception of it: (1) the signal originates on the ocular surface; (2) is relayed to the brainstem; (3) then to the limbic system; and (4) finally to the cerebrum.[77,95] At each step, the signal (and ultimately the perception of ocular discomfort can either be upregulated or downregulated by nociceptive processing in the brainstem, emotional state in the limbic system, memories of pain in the parietal lobe of the cerebrum and the level of attention given to pain in the frontal lobe of the cerebrum, which are all influenced by an individual's biology, psychology, and cultural upbringings.[81,82,96–98] Although the neural processing of pain is complex, research suggests that pain sensitivity, defined as how individuals rate suprathreshold (i.e., painful) stimuli, is the most important metric in understanding how individuals perceive pain.[92,99,100]

Pain sensitivity has been linked with analgesic use after surgery, risk of developing chronic pain, and how successful a medical procedure is perceived.[89,100–103] Experimentally, an individual's pain sensitivity can be measured by: (1) determining the length of time a hand can be placed in ice-cold water, (2) the level of heat tolerated when a thermal stimulus is administered, or (3) the amount of pressure that can be endured when a direct force is applied.[92,99] Using these tests, an individual with higher pain sensitivity would notice pain at a lower stimuli level. The potential of using pain sensitivity to understand ocular discomfort was demonstrated by Vehof et al., but the lack of research in this area may be partially explained by the inherent challenges of measuring pain sensitivity experimentally as it is expensive, time-intensive, requires highly-trained staff and faces the moral quandary of inducing pain in healthy subjects.[92,99]

The Pain Sensitivity Questionnaire (PSQ), which was developed by Ruscheweyh et al. in 2009, may offer a remedy to some of the inherent challenges.[92] The PSQ is a self-rating instrument, taking three to five minutes to complete, which asks individuals to imagine themselves in painful situations that are commonly experienced and rate the pain they feel they would experience from a scale of 0 ("Not at all painful") to 10 ("Most severe pain imaginable") (Fig. 1). The PSQ provides a score that rates pain sensitivity from a scale of 0 to 10, with a higher score associated with greater pain sensitivity; the PSQ has been validated in normal and chronic pain populations.[89,99,104–107] Validation studies have shown that the PSQ scores were significantly correlated with experimental measures of pain sensitivity in normal individuals and chronic pain sufferers.[89,99,104–107] As the PSQ had never been used in ocular surface research, the study described in Chapter 5 of the thesis was done to determine if an individual's PSQ score was associated with how ocular discomfort is perceived.

This dissertation was meant to address the frustration experienced by EDE sufferers regarding lack of treatment options, even after significant resources have been spent on failed clinical trials. This is partially attributed to the lack of a diagnostic test that is accurate, objective, repeatable and associated with symptomology; without such a test, it would be nearly impossible to measure treatment efficacy and prove that a drug should obtain FDA approval.[18,45,47,48]

Chapter 2: Ocular Surface Cooling Corresponds to Tear Film Thinning and Breakup.

Note: This chapter was published under the same title in Optometry and Vision Science. 2015 Sep;92(9):e248-56. The co-authors were Andrew Graham, Steve Selvin and Meng C. Lin; permission to include this material in the dissertation was received from all of them.

2.1 ABSTRACT

Purpose: To investigate the relationship between ocular surface temperature (OST) and tear film thinning and breakup.
Methods: Simultaneous imaging of OST and fluorescein tear thinning and breakup (FTBU) was performed on 20 subjects. Subjects were asked to open their eyes and refrain from blinking for as long as they could during testing. OST was measured using an infrared thermographic camera (FLIR A655sc) and rates of ocular surface cooling (OSC) were analyzed using commercially available software. A method was developed to quantify the rate of FTBU formation using image-processing software.
Results: Areas of FTBU and regions of OSC were observed to be co-localized, with localized cooling preceding the formation of FTBU. The rates of OSC and FTBU formation were positively correlated ($r=0.74$). A 2nd order polynomial model accurately describes the physiological relationship between the area of FTBU and OST ($p<0.001$). A linear approximation provides a more clinically interpretable rate of FTBU formation with decreasing OST ($p<0.001$), while still retaining high R^2.
Conclusions: The results suggest a direct relationship between FTBU formation and OSC. That cooling of the ocular surface precedes FTBU formation implies a process of evaporation contributing to tear film thinning and breakup. Our study suggests that measuring the OSC rate could be an indirect assessment of tear evaporation, and could contribute to the management of evaporative dry eye.

2.2 INTRODUCTION

The International Dry Eye Workshop in 2007 identified aqueous evaporation as the leading cause of dry eye (DE), and evaporative dry eye (EDE) as the most prevalent type of this widespread disease.[18] Although EDE has a high prevalence and creates a significant economic burden, there is still a limited understanding of how evaporation of the tear film contributes to the disease process.[1,2,17,108] The etiology of EDE has been investigated through mathematical models, but there has been a general lack of clinical work done to confirm the models, due to limitations in the tools available to clinically assess tear evaporation.[23,25] This gap in knowledge has served as an impetus for clinical investigators to develop new ways to assess tear film evaporation; one promising approach is the use of a thermographic camera (TC) to measure ocular surface temperature (OST).[55,109–111]

Mapstone first adapted the TC to measure the temperature of the ocular surface, and other researchers later implemented the technique to investigate the role of OST in DE.[52,53] Recent research has focused on using the TC to measure tear film evaporation indirectly, based on the theory that when tear evaporation occurs, the phase change

from liquid to gas is associated with heat transfer to the surrounding environment, and thus a cooling of the ocular surface.[54–59] It is known that liquids with a higher rate of evaporation evince a greater rate of surface cooling; as an example, when ethanol and water are applied to the skin, the area treated with ethanol will decrease in surface temperature more rapidly than the area with water applied, because the rate of evaporation is higher for ethanol.[60,61] It is reasonable to hypothesize that tear evaporation leading to tear film thinning and breakup should be associated with concomitant ocular surface cooling (OSC).[28,54,55,62] Furthermore, the OSC rate should reflect the rate of tear evaporation, and thus be directly related to the rate of tear thinning and breakup.[59–61] These relationships, however, have not been conclusively demonstrated to date, as some studies have reported an association between OST and tear film stability, while others have found no such association.[56,58,63–66] A possible reason for the conflicting results is that most studies have measured OST and tear film stability separately – a significant issue given the highly variable and dynamic nature of the tear film.[54,56,57,63,67–69]

In the current study, our aim is to develop a methodology to investigate the OSC during the inter-blink period concomitantly with a quantitative measure of fluorescein tear break-up (FTBU). We will develop a statistical model describing the increase in the area of the ocular surface exhibiting FTBU as a function of the decrease in OST. We will also present a linear approximation to this model that will provide clinicians with an easily interpretable rate of FTBU formation as a function of OST. In addition to improving our understanding of the physiology of tear thinning and breakup, such an indirect measure of tear evaporation that can be performed concomitantly with standard imaging of FTBU could be a useful clinical tool in pharmacological management of DE. Finally, it has been suggested that FTBU and OSC play a central role in the etiology of symptoms associated with EDE, and this study may help us better to understand this relationship.[26,76]

2.3 METHODS

2.3.1 Subjects

Subjects were recruited from the University of California, Berkeley School of Optometry. Subjects taking systemic or ocular medication, or with a history of ocular disease or surgery were excluded from the study. Subjects were instructed to refrain from using any eyelid makeup or eye drops on the day of the visit. Informed consent, with a complete description of the goals, risks, benefits and procedures of the study, was obtained from all participants. This study observed the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley Committee for Protection of Human Subjects. A full slit lamp examination was performed at the beginning of the visit to ensure that no sign of ocular surface disease was present.

2.3.2 Instrumentation and Procedures

OST was measured using the FLIR A655sc (FLIR Systems, Inc., Wilsonville, OR, USA), an uncooled microbolometer TC with a 640x480 video resolution, 17 μm pixel size, and 0.1°C thermal sensitivity. A digital video camera (DXC390 3CCD Exwave HAD, Sony Electronics, Inc., Tokyo, Japan) attached to a slit lamp (SL 120, Carl Zeiss

Meditec AG, Jena, Germany) was used to record FTBU. The TC, mounted on a tripod, was placed behind the slit lamp at a distance of 16-18 inches from the eye, aligned approximately 15 degrees off-axis temporally from the geometric center of the cornea, while the optical system of the slit lamp and digital video camera was aligned approximately 15 degrees off-axis nasally (Figure 2-1). FTBU was assessed under cobalt blue illumination and viewed through a 530 nm yellow barrier filter.

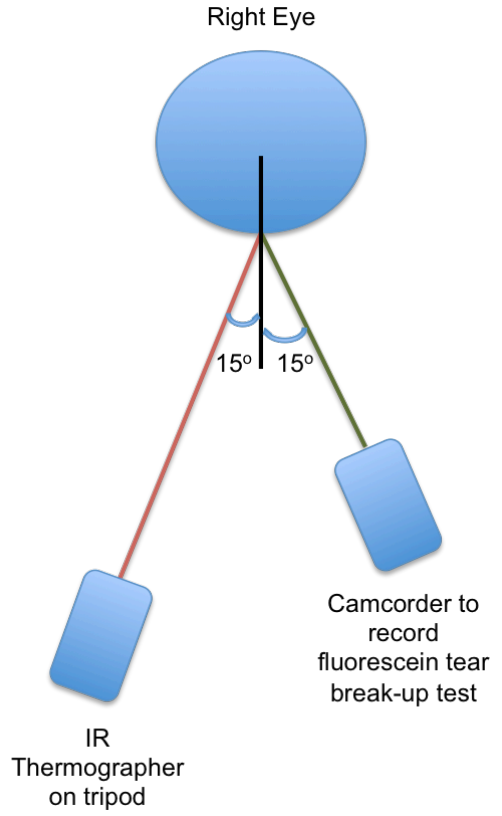

Figure 2-1 Placement of the thermographic camera and the slit lamp relative to the subject's eye.

Subjects were asked to complete an Ocular Surface Disease Index (OSDI) questionnaire prior to measurements. All measurements were taken in an examination room and subjects were acclimated to the ambient environment for a minimum of 10 minutes before testing.[64] A micropipette was used to instill 4 μl of 2% sodium fluorescein dye onto the superior bulbar conjunctiva and subjects were instructed to close and roll their eyes to evenly distribute the dye. Subjects were then positioned at the slit lamp, and the slit lamp and the TC were focused on the right eye. Subjects were instructed to blink 5 times and then to refrain from blinking or moving their eyes for as long as possible, in order to maximize the observation of FTBU and OSC, while the digital video camera and TC simultaneously imaged the ocular surface. Three such trials were conducted in sequence, each trial separated by 20 sec of eye closure to allow the tear film to recover.

The video sequences from the OST and FTBU recordings were synchronized using Final Cut Pro X (Apple Inc., Cupertino, CA, USA). The FLIR+ Tools software suite was used by an experienced observer (WL) to specify a user-defined region of interest in the TC images corresponding to the cornea; the region represented 4000-7000 measurement points (depending on anatomical variation), with the mean value of the points interpreted as the average ocular surface temperature.[55] Image-processing software (NI LabVIEW Vision Assistant 2012, National Instruments Corp., Austin, TX, USA) was used to isolate the imaged area of the cornea, after which the full-color videos were split into the red, green, and blue channels. The green channel, which provided the best imaging of FTBU, was then converted to 8-bit gray scale with 256 levels of luminance.

In this study, we utilized a fluorescein video sequence to capture the process of FTBU, which began with some areas losing fluorescence and beginning to darken, and in some cases culminated in complete disruption of the tear film. Our method set a pixel threshold luminance value above which a pixel was considered “bright” and represented an intact area of tear film, and below which was considered “dark” and represented an area of tear film that was undergoing FTBU, presumably through evaporative thinning (Figure 2-2).[29,112] The principles for FTBU quantification employed by this study were drawn from previous research in which a threshold luminance value was subjectively chosen that most closely approximated the pattern of FTBU formation observed.[113] To mitigate the subjective nature of the assessment, the threshold values we determined for all subjects were averaged to obtain a mean threshold value used uniformly for FTBU quantification in all subjects. The quantification provided the proportion of the corneal image exhibiting FTBU (i.e., the proportion of “dark” pixels) over the time course of each trial, which was then compared to the mean OST from the TC recording, over the same, synchronized time sequence.

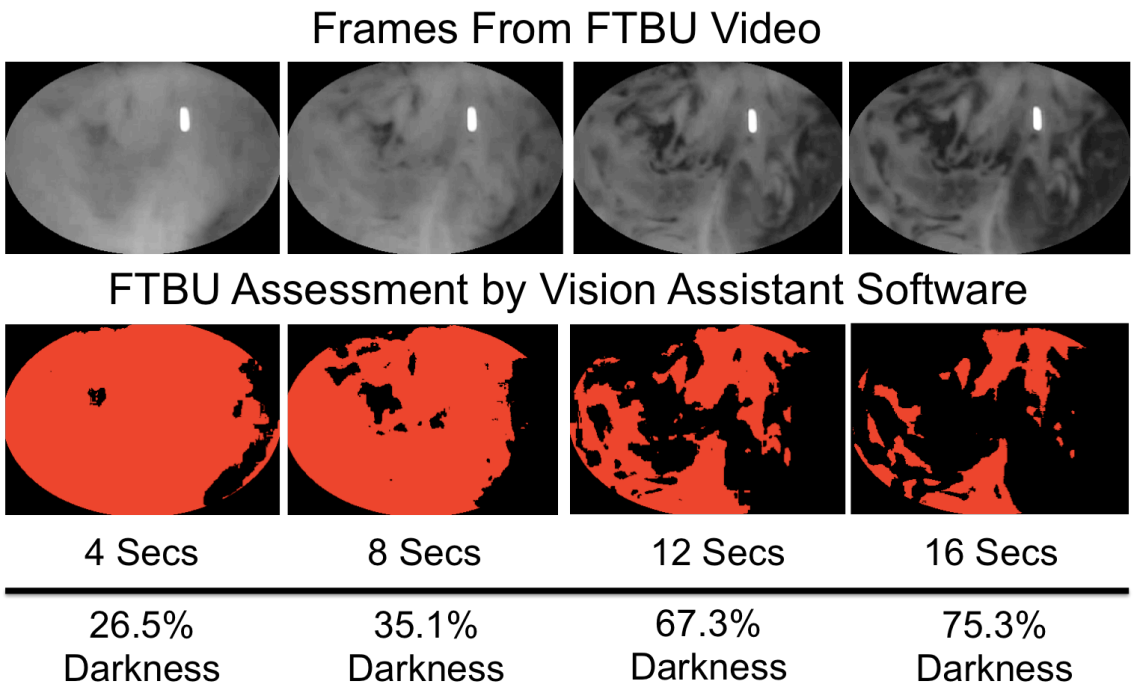

Figure 2-2 A threshold value was subjectively selected that would most accurately reflect FTBU seen for each subjects. The threshold values were averaged and used for FTBU quantification.

2.3.3 Statistical Methods

For each subject, it was first verified that FTBU (as represented by the proportion of sub-luminance threshold pixels) increased and OST decreased over the time course of the trial while the subject refrained from blinking, and that their respective rates of change were directly correlated. As it turned out, for reasons detailed below, 20 of 25 subjects showed a clear trend of decreasing OST and increasing FTBU over time. A type of mixed effects repeated measures model, referred to as a "random intercepts model", was fit to the aggregate data from all 20 subjects who exhibited FTBU and OST changes over time. The most physiologically accurate model of FTBU was determined to be a 2^{nd} order polynomial function of OST in the fixed effects, with each subject having an individual random offset to the population average estimated intercept. This type of model has an advantage for this study in that the threshold luminance we used to distinguish dark from bright pixels was a group average, resulting in the first image frames for all subjects having somewhat different starting proportions of dark pixels, which individual variation in starting values was reflected in the random intercept estimates. We also fit linear approximations to these quadratic models, because the linear regression slope is a more clinically-interpretable measure of the rate of FTBU with decreasing temperature, and these models did retain acceptable fit statistics (e.g., high R^2).

2.4 RESULTS

Twenty-five subjects (22 female, 3 male), with a mean (SD) age of 21.2 (2.4) years and a range of 18 to 27 years, completed the study. Five subjects were unable to provide usable data: two subjects (1 Asian female, 1 Asian male) were unable to hold their eyes steady and open without blinking and could not provide usable images; one subject (Caucasian female) had a partial blink in the middle of the measurement period; one subject (Latino female) was unable to open the eye wide enough to prevent the eyelashes from producing artifacts in the images; one subject's (Asian female) images suffered from low exposure, possibly due to insufficient fluorescein loading in the presence of reflex tearing. Twenty subjects successfully completed the study and provided data for analysis.

2.4.1 OSC and FTBU: Qualitative Observations

A review of the synchronized digital video recordings showed that a majority of subjects exhibited areas of OSC and FTBU formation that were located in the same region and presented with similar patterns (Figure 2-3). When regions of OSC and FTBU were co-localized, cooling was always noted 1 to 2 seconds before an observable area of FTBU; FTBU never occurred unless OSC preceded it (Figure 2-4). The agreement between OSC and FTBU was most common during the first trial and became less common with each subsequent trial, which was usually associated with an increase in the height of the lacrimal tear lake and often increased reflex tearing (Figure 2-5). For this reason, subsequent quantitative analyses focused on the first trial period only. Note that in trials in which OSC and FTBU failed to coincide, either only OSC was observed without FTBU, or neither OSC nor FTBU were detected (Figure 2-5).

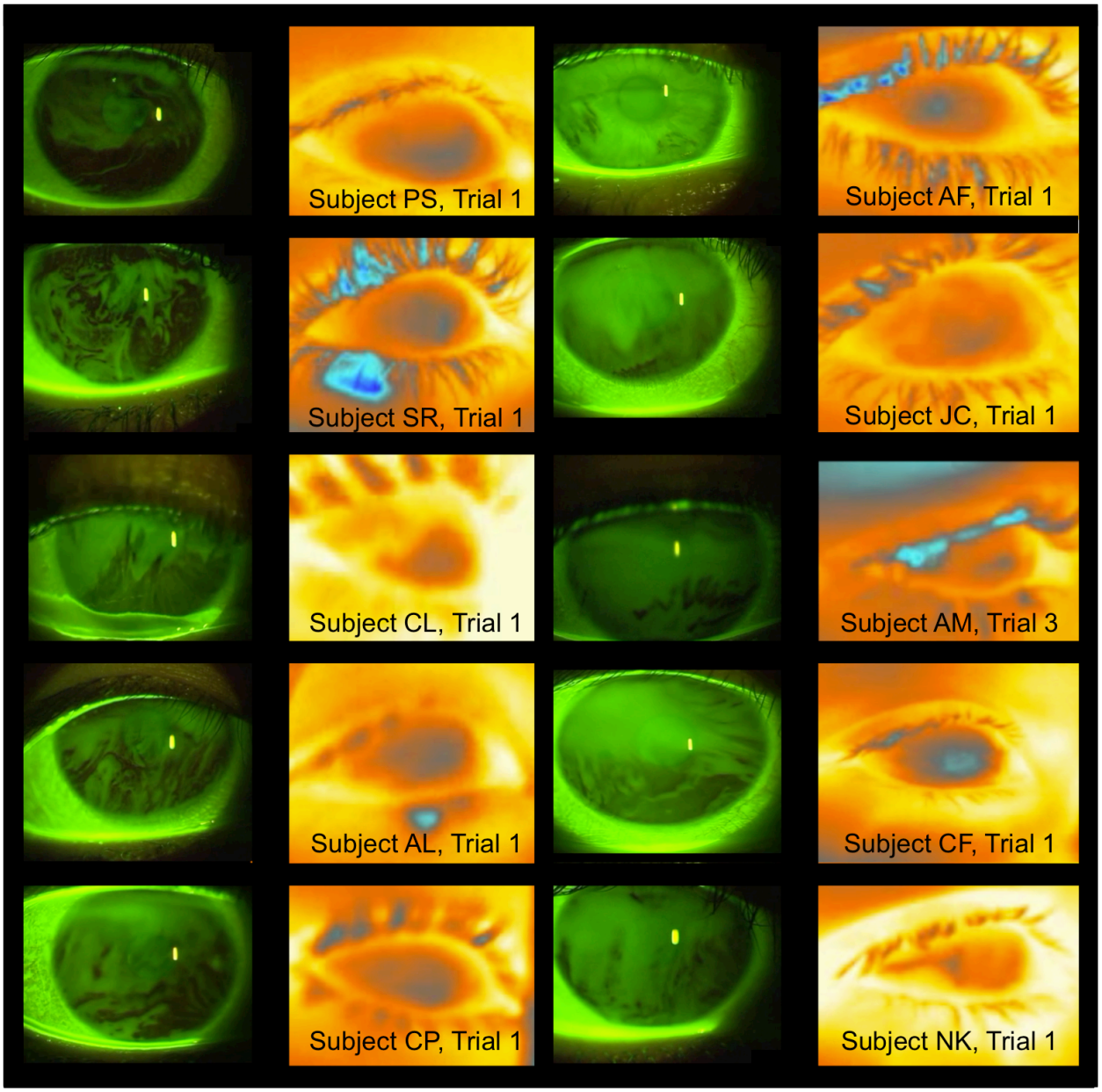

Figure 2-3 Screen captures taken from synchronized OST and FTBU video recordings shortly before subjects blinked. Areas of OSC (blue regions) appeared to coincide with regions of FTBU when examined at the same time point.

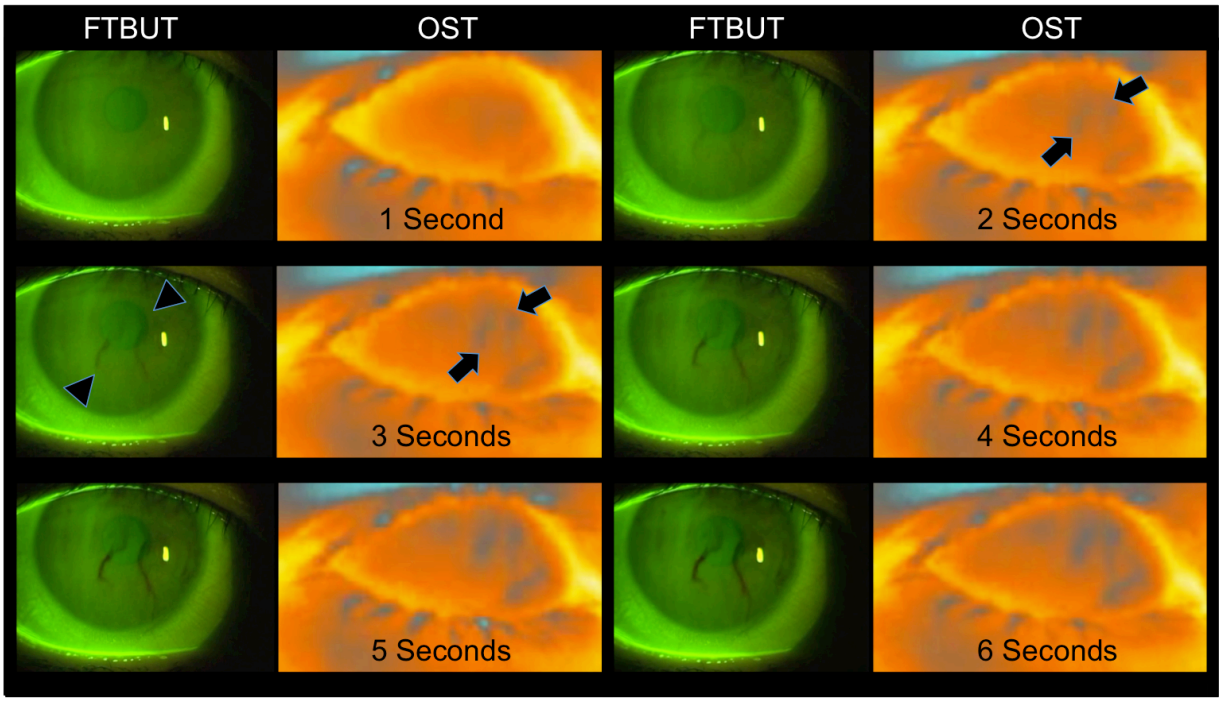

Figure 2-4 Time-lapse sequence of a subject's synchronized recordings, areas of cooling (arrows) were identified at two seconds, while corresponding areas of FTBU formation (arrowhead) appeared approximately 1 second later.

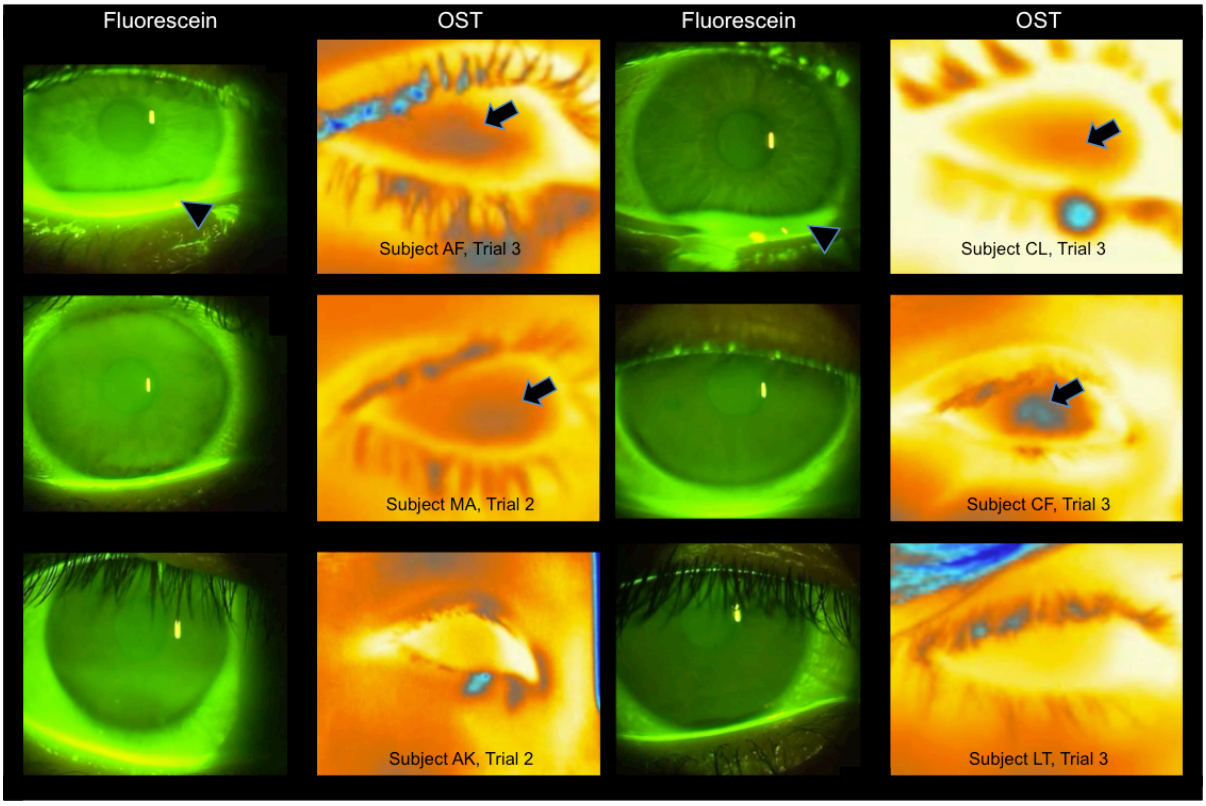

Figure 2-5 Subjects AF, CL, MA and CF exhibited OSC (arrows) without any obvious FTBU formation. Subjects AF and CL had increased lacrimal tear lake height

(arrowhead). Subjects AK and LT showed no evidence of OSC or FTBU formation. Although OSC could occur in such cases without FTBU, in no case did FTBU occur without OSC.

2.4.2 OST and FTBU: Quantitative Analysis

Subjects were able to hold their eyes open without blinking for a mean (SD) time of 15.50 (10.27) sec. During the first inter-blink period, subjects averaged a 33.6% increase in corneal surface area with FTBU (defined as the proportion of pixels in the fluorescein images that were below the luminance threshold, as described in detail above). The mean (SD) baseline OST after the first blink was 35.2 (0.4) °C, and during the first inter-blink period, OST decreased by a mean (SD) of 0.80 (0.47) °C. Figure 2-6 shows the decrease in OST during the first inter-blink period for a typical subject, and Figure 2-7 shows the increase in the corneal image area exhibiting FTBU for the same subject over the same time period, both with regression lines shown.

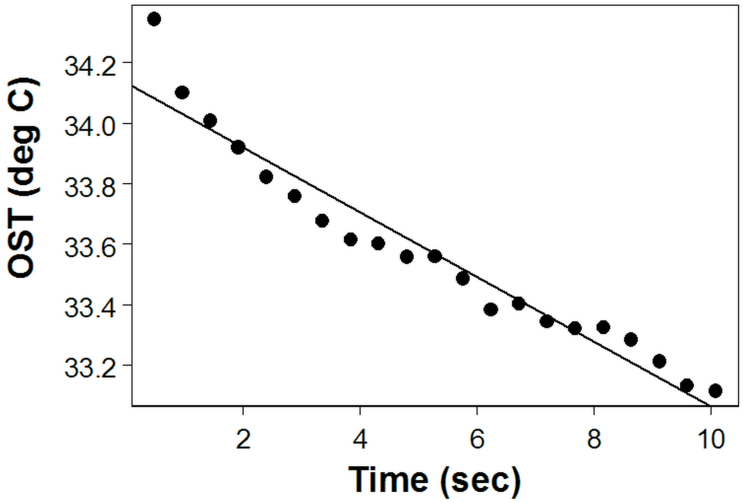

Figure 2-6 Decrease in OST during 1 inter-blink period for a typical subject. Concomitant increase in FTBU for the same subject, same trial, shown in Figure 6.

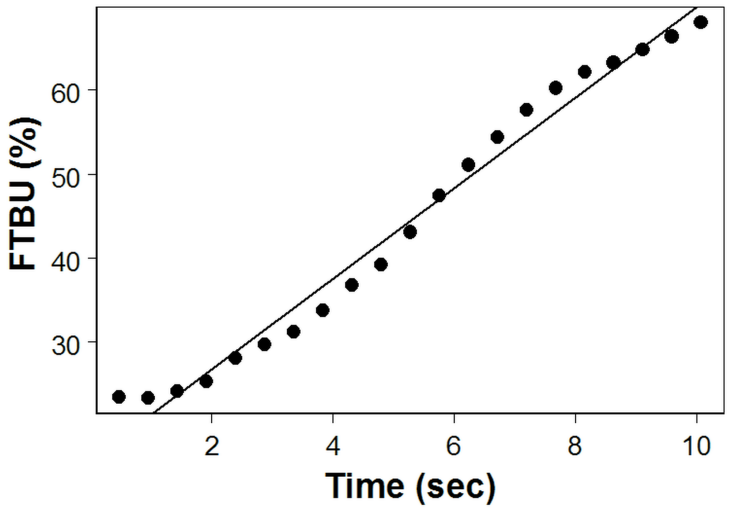

Figure 2-7 Increase in % of image displaying FTBU during 1 inter-blink period for a typical subject. Concomitant decrease in OST for the same subject, same trial, shown in Figure 2-5. FTBU at time 0 is calculated as approximately 25% due to the use of a group-averaged threshold luminance value to quantify FTBU, and from image artifacts (e.g., eyelash) that were interpreted by the software as “dark”.

The mean (SD) OSC rate was -0.057 (0.036) °C/sec, and the mean FTBU formation rate, which describes the proportion of the image of the corneal surface with tear breakup or thinning developing over time, was 3.1%/sec. The rates of OSC and FTBU formation showed a relatively strong positive correlation ($r = 0.74$), with a higher rate of cooling associated with a faster rate of FTBU formation (Figure 2-8). Taking each subject’s linear FTBU and OSC rates, we can see from the figure that a faster rate of cooling at the ocular surface is significantly related to faster FTBU formation ($p < 0.001$).

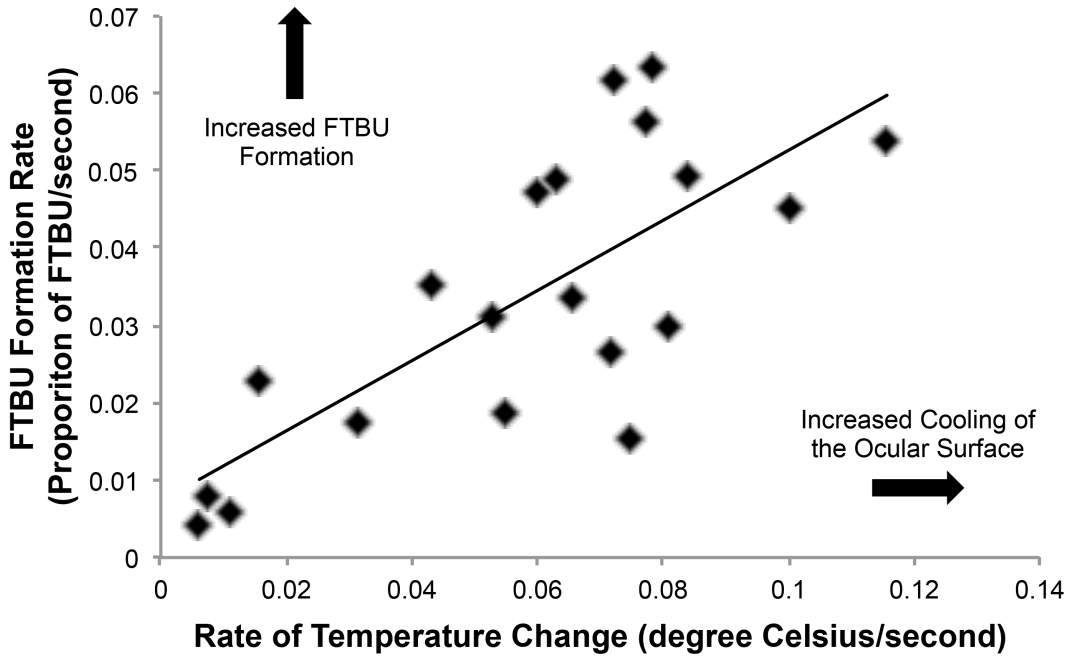

Figure 2-8 OSC rate and FTBU rate from the synchronized thermographic and fluorescein recordings. A faster rate of evaporative surface cooling is associated with a faster formation of FTBU.

Figure 2-9, 2-10 shows the direct relationship between FTBU and OST, with data from all subjects modeled as 2nd order polynomials. Model fit statistics were uniformly good, with R^2 ranging from 0.77 to 0.99, with a median R^2 of 0.98 across all subjects. We examined various models of FTBU as a function of OST, using the aggregated data from all 20 subjects. We found the best fitting model to be a random intercepts model, having fixed effects of:

$$\mathbf{FTBU = 82.201 - 4.195*OST + 0.053*OST^2}$$

with p-values for all 3 coefficients being < 0.001. In this random intercepts model, each subject was considered to have an additional random offset from the population average estimated intercept. Figure 2-10 supports this model, as it shows that subjects had approximately the same shape of upward-trending FTBU curve with decreasing OST, but there were individual variations in the initial proportion of pixels classified as sub-threshold immediately upon opening the eye after the first blink. Starting proportions ranged from 21% to 44% of pixels, which was due, in part, to the use of a group-averaged threshold luminance value, and in part to inter-subject differences in palpebral aperture size and in the extent to which lashes created “dark” artifacts at the edges of the corneal images.

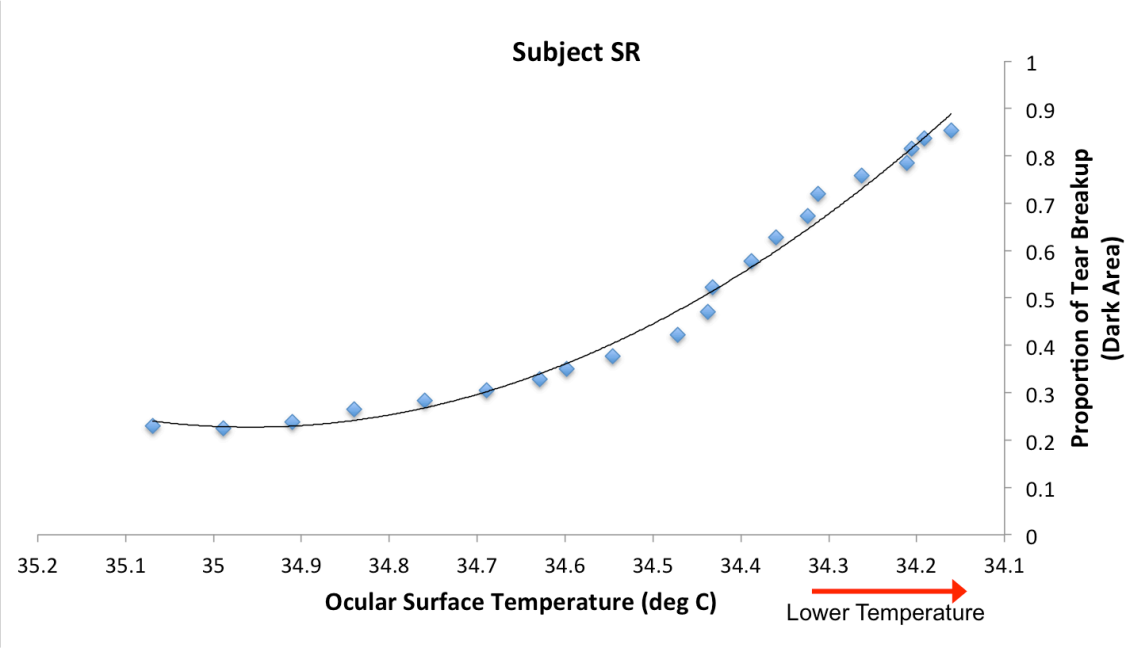

Figure 2-9 OST and FTBU were plotted from the same time points for Subject SR. Cooling of the ocular surface appeared to be associated with increased tear breakup.

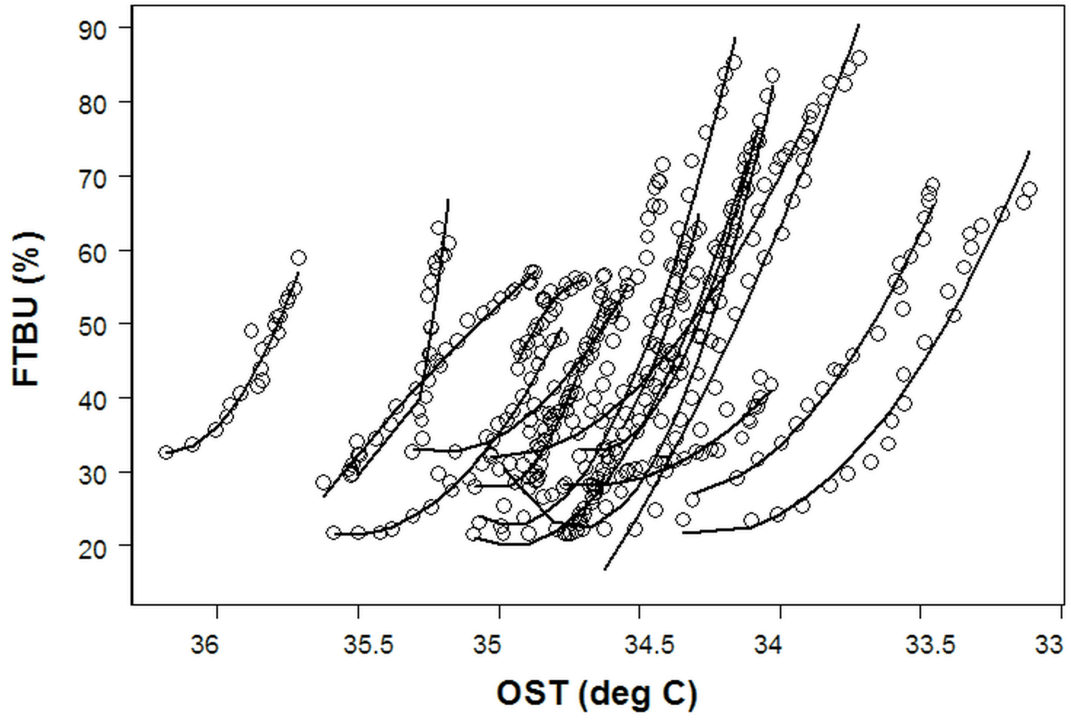

Figure 2-10 FTBU as a function of OST, with individual 2nd order polynomial mixed effects model fits for each subject. An increased rate of OSC was associated with a higher rate of FTBU formation.

Although the 2nd order polynomial model was the most accurate in terms of the physiological process of evaporative tear thinning and breakup, we also found that linear approximations to these curves retained good fit statistics (e.g., R^2 ranging from 0.63 to 0.99, with a median R^2 of 0.88 across all subjects) and would be more clinically interpretable: the linear regression slope is an estimate of the rate of FTBU formation per unit OST decrease. The mean (SD) slope across all 20 subjects was 0.604 (0.222) and ranged from 0.201 to 1.122. The interpretation is that, on average, based on our study cohort and the methodology (e.g. volume of fluorescein instilled), the corneal area exhibiting FTBU increases approximately 60% per degree of OST decrease. The mean OST decrease during the first inter-blink period was 0.8°C, corresponding to an approximately 48% increase in the corneal area exhibiting FTBU. This number appears high by clinical standards because of the stress test nature of this experiment, in which subjects held their eyes open as long as possible, even after the onset of FTBU would normally have stimulated blinking. Further study is needed to determine the amount of OSC and FTBU (as quantified by this method) experienced by patients in a normal setting.

2.5 DISCUSSION

Cooling of the ocular surface has been attributed to evaporation of the tear film, convective heat transfer, and the emission of infrared radiation.[56,114–116] Fluorescein tear breakup has been attributed to lipid migration and dewetting of the cornea, rupture instability by Hamaker dispersion forces, surface-tension gradient instability, tear rupture due to mucin breakup, evaporation of the tear film, inflow of tears into the cornea, and tangential flow from the displacement of sodium fluorescein particles.[25,28,32,62] The common factor for OSC and FTBU appears to be the evaporation of the tear film.[25] This study provides evidence that OSC and FTBU are associated, and suggests that a common physical force such as tear film evaporation is acting in both processes, which is in agreement with a previous study that also simultaneously assessed FTBU and OST.[66] Unlike the study by Su et al., which was specifically focused on demonstrating that areas of OSC and FTBU were co-localized, this study examined how OSC (as a proxy for evaporation) influenced FTBU formation over the time course of the inter-blink period.

Two subjects demonstrated signs of reflex tearing (likely due to ocular irritation from not blinking), evidenced by the significant increase in the lacrimal tear lake height seen during testing (Figure 2-5). In these cases, FTBU was not observed but OSC was still detected, which suggests that these two measurements assess different aspects of tear film evaporation.[117,118] This argument is strengthened by the observation that OSC always preceded the formation of FTBU, implying that OST measurement was indirectly assessing the active process of tear film evaporation while FTBU was an end point resulting from a tear film that is thinned enough by evaporation to cause decreased fluorescence intensity or quenching.[62] Since evaporation of the tear film still occurs during reflex tearing, it would explain how OSC was observed without FTBU in these two subjects.

The results of this study are consistent with a mathematical model developed by Peng et al., which postulates a mechanism by which local rupture of the tear film lipid layer (TFLL) increases local tear evaporation rate leading to tear-film rupture and FTBU.[25] In this model, an intact, thick TFLL decreases tear evaporation rate. Thus, a prerequisite for FTBU formation is an area of tear lipid layer deficiency (i.e. rupture) that increases evaporation rate, which in turn is associated with ocular surface cooling.[54–61] Local high evaporation rate drives a deepening rupture spot in the tear film, which when sufficiently thin enough can exhibit FTBU (Figure 2-11).[62] The time necessary for the tear rupture spot to evaporate towards FTBU explains the lag time observed between OSC and FTBU, and is in agreement with the evaporative tear breakup model in which local tear cooling always occurs before areas of FTBU are observed.[25]

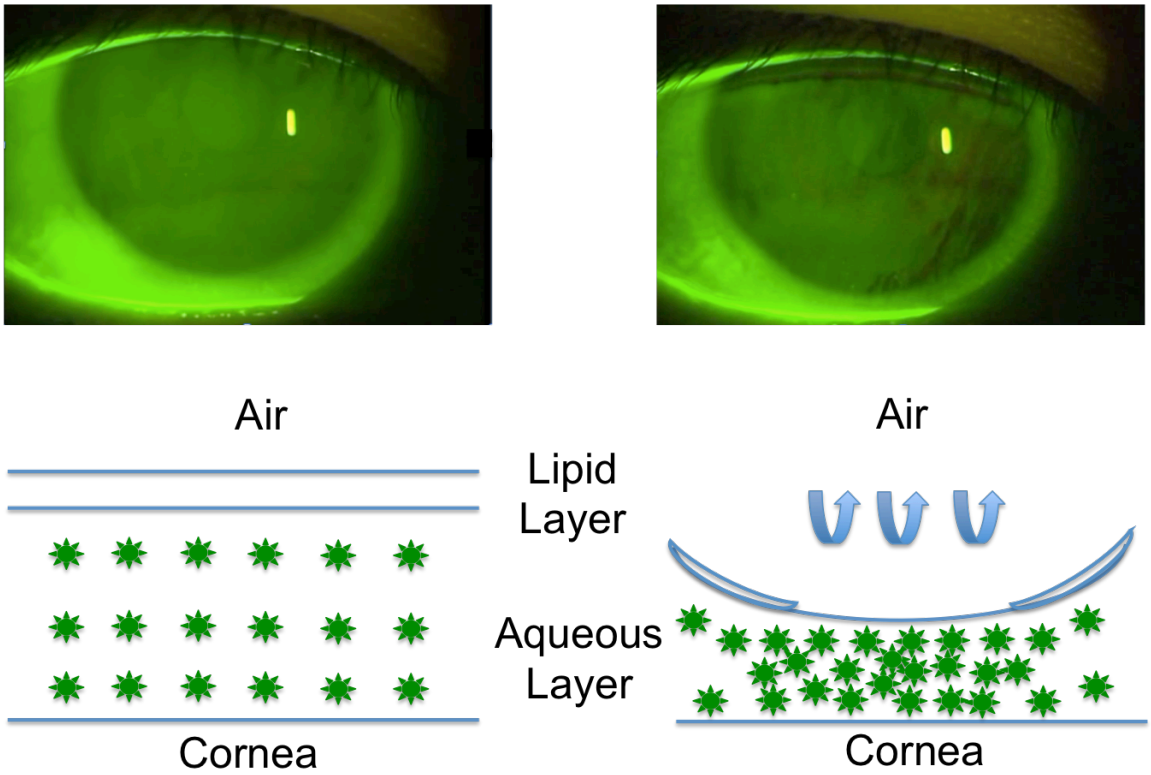

Figure 2-11 Rupture of the TFLL rupture causes evaporation (observed as OSC) and with enough evaporation leads to subsequent fluorescein quenching.

It is interesting to note that OSC and FTBU have both been linked to symptoms associated with EDE.[26,76] In a post-hoc analysis, we found no association between the rates of OSC or FTBU formation and OSDI score; however, a borderline-significant association ($p=0.060$) was found between OSDI score and the slopes of the linear approximations to the individual regression curves of FTBU on OST. Although not reaching statistical significance, this post-hoc model suggests that subjects with a faster rate of FTBU formation per unit temperature decrease had, on average, higher OSDI scores. Post-hoc power analysis based on statistical simulations was performed to examine how increasing the sample size would affect the statistical significance between OSDI and the rate of FTBU formation per unit temperature decrease. In our

simulations, doubling the sample size to 40 subjects resulted in a statistical significance less than 0.05; however, it is difficult to make a definite statement about the association due to our small study cohort.

We also speculate that the slope of the regression curve of FTBU on OST (i.e., how fast the tear film thins and breaks up for a given amount of temperature decrease) could provide a measure of how sensitive a patient's tear film is to temperature change. It is conceivable that this measure could provide greater insight into the symptoms experienced by EDE sufferers than either the OSC rate or FTBU formation rate alone. Assuming that OSC represents the active process of evaporation, then the slope of the regression of FTBU on OST could provide information on the level of evaporative stress the tear film can withstand before breakup occurs. It is thought that individuals with EDE are more susceptible to evaporation of the tear film, possibly due to a deficient tear film lipid layer, which in turn is thought to cause hyperosmotic stress associated with EDE symptoms.[23,26,32,119,120] An individual with EDE may have a tear film that can withstand less evaporative stress before FTBU is noted (i.e., a steeper slope, or faster rate of FTBU per unit temperature decrease) compared to an individual without EDE who may have a slower rate. Further investigation is warranted to test this hypothesis and to understand how the sensitivity of the tear film to evaporative stress varies among individuals with and without EDE.

Though this study suggests there is potential in simultaneously examining OST and FTBU as a clinical tool, issues were also noted during the study that suggest that additional work is needed to refine the technique and to demonstrate its efficacy in the assessment of EDE. One issue was related to the volume of fluorescein instilled in the eye. Four μl is within the commonly accepted range of volume reported in various clinical studies,[47] but is on the high end of this range. This volume was selected to maximize the chances that sufficient fluorescein would be present during the second and third trials. Various studies have suggested that the volume of fluorescein instilled could alter tear film stability, which may have artificially influenced the rate of FTBU formation.[32,117] Future work should likely limit the fluorescein instilled to 1 to 2 μl to decrease the possible confounding influence of fluorescein volume on the rate of FTBU formation. Another issue arose in cases in which FTBU was not observed, such as the examples in Figure 4. These cases were predominantly noted upon the second or third trial so there is a possibility that the concentration of fluorescein decreased to a point where FTBU was difficult to see.[32] It should be noted that a relatively large volume of fluorescein was instilled into the eye in order to minimize the possibility of this occurring. Nevertheless, it is impossible to determine if a low fluorescein concentration or a lack of FTBU formation contributed to the occasional inability to observe FTBU.

In future work, we intend to explore possible methods for quantifying the extent to which co-localization occurs, and to implement an automated algorithm for setting the luminance threshold. Finally, a larger sample size is warranted to determine the clinical value of assessing the rate of FTBU per unit temperature decrease, and how this metric is associated with OSDI score by investigating how it differs in cohorts with and without EDE signs and symptoms.

2.6 CONCLUSIONS

By simultaneously assessing FTBU and OST, we were able to show that localized areas of OSC represent regions of elevated evaporation. For the foreseeable future, FTBU will be more commonly used in clinic to assess evaporation as it is more readily available and provides more spatial detail. Nevertheless, measuring OST will likely become more common as it is more objective, more easily interpretable (as an OSC rate) and allows for evaporation assessment without disrupting the tear film. This holds important implications for the clinical evaluation of EDE due to our current inability to accurately assess tear evaporation *in vivo*. The use of a TC may be the best method available today to indirectly measure tear evaporation rate, which may lead to improvements in the diagnosis, management and treatment of evaporative dry eye.

Preface to Chapter 3

Our published study, which simultaneous assessed OST and fluorescein tear breakup (FTBU), demonstrated that areas of FTBU and regions of cooling were co-localized, and that cooling was noted one to two seconds before an area of FTBU was observed. This implies that OST measurement indirectly assessed the active process of tear film evaporation while FTBU was an end point resulting from a tear film that is thinned enough by evaporation to cause fluorescence quenching.[32,62,117,118,121] The results are consistent with a mathematical model developed by Peng et al., which theorized an intact TFLL would decrease TER.[25] Therefore, a requirement for FTBU formation is an area of TFLL breakup that increases TER, which is observed as an area of cooling. The locally elevated evaporation rate causes a deepening fissure in the tear film, which when sufficiently thin causes FTBU.[25] The results from the study suggest that there is significant potential for IRT to be a gold standard test to diagnose and monitor EDE in patients.

Nevertheless, more information must be gained about the IRT's potential before it is employed in research and clinical settings. One important area that has been ill defined is the test-retest repeatability of IRT in the measure of OST and OSC rate. There have been two studies that have examined this issue and they examined aspects of repeatability that likely has limited value in understanding its clinical potential. The first study was done by Klamann et al., which reported an intraclass correlation coefficient (ICC) of 0.95 from three consecutive measurements done in one visit for the temperature of the ocular surface.[110] Though that is an extremely high ICC value, it is important to note that they were only looking at the temperature of the ocular surface when the eyes opened after 5 seconds of closure. This value has minimal value as the OST, when the eye is first opened, is close to core body temperature and does not offer any insights that would help diagnose or monitor EDE. The second study, which was done by Petznick et al. also showed high repeatability for OST measurements 0.93-0.97 but they were hampered by a small sample size (16 subjects) and they similarly only examined the temperature of the ocular surface.[67] For IRT to be clinically useful in the diagnosis and monitoring of EDE, a profile on inter-day and intra-day repeatability must be determined, which was the purpose of next study.

Chapter 3: Repeatability of Ocular Surface Cooling Measurement

3.1 ABSTRACT

Purpose: Measuring ocular surface cooling (OSC) rate indirectly measures tear evaporation rate and may serve as an important tool to diagnose evaporative dry eye (EDE). Nevertheless, the clinical potential of measuring OSC rate is unknown, as its repeatability profile has not been established.
Methods: Prior to each measurement, subjects were acclimated to the environment for 10 minutes. Subjects were asked to close their eyes for two minutes and then open their eyes and refrain from blinking for as long as possible while OSC rate was measured using an infrared thermographer (IRT). Subjects were seen for two visits, with the visits separated by at least one week; morning (AM) and afternoon (PM) measurements were taken at each visit. Intra- and inter-day repeatability was assessed using Intraclass Correlation Coefficient (ICC). Intra-day ICC was calculated from the AM and PM measurements. Inter-day AM and PM ICC were calculated separately for the two AM and two PM measurements, respectively. Subjects were brought in for a third visit for a fluorescein tear breakup time (FTBUT) assessment; they were categorized as having EDE if they had a FTBUT < 5 seconds in one eye.
Results: Forty-two subjects (36 females and 6 males) completed the study. Nineteen subjects were categorized with EDE and twenty-three subjects as normal; they had similar baseline characteristics. OSC rate for subjects with EDE (0.10 ± 0.06^0C/sec) was significantly greater than for subjects without EDE (0.07 ± 0.05^0C/sec); linear mixed effects model (p=0.04). Intra-day ICC ranged from 0.24 – 0.49 and was similar between EDE and normal. Inter-day ICC was greater for EDE subjects (range: 0.61-0.85) than for normal subjects (range: 0.27-0.57).
Conclusions: OSC rate was higher in EDE than in normal subjects. OSC measurements demonstrated relatively poor intra-day repeatability but fair to good inter-day repeatability. Inter-day repeatability was better in EDE, which suggests that IRT may be useful in monitoring EDE.

3.2 INTRODUCTION

Dry eye disease is the most common ocular morbidity in the world, thought to affect 5-30% of the world population; with sufferers often reporting symptoms of ocular irritation, fatigue, photophobia, excessive tearing, and redness.[1–5] Severe dry eye can lead to permanent sight loss but even mild to moderate dry eye is associated with a decreased quality of life and increased risk for depression.[9–14] In addition, the annual economic cost of dry eye in the United States is estimated to be 55.4 billion dollars.[17] The International Dry Eye Workshop in 2007 identified evaporative dry eye (EDE) as a prevalent type of dry eye.[19] Given its importance, it is surprising that there has yet to be a gold-standard test developed to diagnose and monitor EDE.

This has led researchers to recently identify infrared thermography (IRT) as a viable non-invasive technique for indirectly measuring tear evaporation rate, with greater OSC rate representing higher evaporation.[54,55,67] During each inter-blink period,

ocular surface temperature (OST) decreases; the ocular surface cooling (OSC) observed is presumably due to evaporation of the tear-film and heat loss to the environment.[54,55,57–59] Previous studies inferred that increased tear film instability, likely caused by higher evaporation, would correspond to greater OSC rate, but this relationship was not conclusively demonstrated until our study showed that they were related.[55,56,58,122] In addition, we determined that localized areas of increased OSC represented regions of tear film lipid layer rupture.[122]

As the tear film lipid layer is thought to inhibit evaporation by approximately 75-90%, the site of a rupture is likely where significant tear hyperosmolarity (theorized to be >600 mOsm) occurs. This is important as animal *in vivo* models have identified transient receptor potential cation channel, subfamily M, member 8 (TRPM-8) as the class of nociceptors likely responsible for symptoms experienced during EDE.[33,71–74] TRPM-8 are activated by hyperosmolarity or cooling; a synergistic activation is observed when both stimuli are applied.[33,75] IRT provides the ability to measure (directly or indirectly) both stimuli, which when combined with its non-invasiveness and objectiveness demonstrates the potential of IRT to be a gold-standard test for EDE. Nevertheless, there has been no work done to define the repeatability of measuring OSC rate.[109,110,123] For it to be clinically useful in the diagnosis and monitoring of EDE, profiles on inter-day and intra-day repeatability must be determined, which is the purpose of this study.

3.3 METHODS

3.3.1 Subjects

Subjects were recruited from the University of California, Berkeley and the surrounding community. Subjects taking systemic or ocular medication, or with a history of ocular disease or surgery were excluded from the study. Subjects were initially recruited based on symptoms related to contact lens wear but upon additional thought, it was determined that categorizing them based on EDE status would be better suited to understand the repeatability of OSC measurements. Subjects were instructed to refrain from using any eye makeup or eye drops on the day of the visit. Subjects were asked to discontinue contact lens wear for at least 24 hours before the appointment. Informed consent, with a complete description of the goals, risks, benefits, and procedures of the study, was obtained from all participants. This study observed the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley Committee for Protection of Human Subjects.

3.3.2 Instrumentation and Procedures

Ocular surface temperature was measured using a FLIR A655sc (FLIR Systems, Inc., Wilsonville, OR, USA) uncooled microbolometer IRT, which has a 640x480 video resolution, 17 µm pixel size, and 0.1°C thermal sensitivity. The IRT, mounted on a tripod, was placed eight to ten inches from the eye, focused on the ocular surface and aligned approximately perpendicular to geometric center of the cornea. FLIR+ Tools software (FLIR Systems, Inc., Wilsonville, OR, USA) was used by an experienced observer (WL) to specify a user-defined region of interest corresponding to the cornea

in infrared recordings. The region represented 4,000–7,000 measurement points (accounting for anatomical variation in palpebral aperture size), with mean value of points interpreted as mean OST and used to calculate a linear OSC rate, degree Celsius per second (^{0}C/sec).[55] Due to the correlation between OST and core body temperature, subject core temperature was measured using an Exergen temporal artery thermometer (Exergen Corporation, Watertown, MA, USA).[124] Room temperature and humidity were measured using a digital thermometer (General Tools & Instruments, Secaucus, NJ, USA).

An anterior segment examination under white light was performed to ensure there was no active or pre-existing ocular pathology (e.g., infiltrates, excessive corneal epithelial irritation). Subjects were taken to another room where they were acclimated to the ambient environment for a minimal of ten minutes before testing.[64] They were placed in a slit lamp head- and chinrest assembly, which minimized head movement during OST recording. Subjects were asked to close their eyes for two minutes to get the OST close to core body temperature. They were then instructed to open their eyes and refrain from blinking or moving their eyes for as long as possible, even if they experienced discomfort, while OST was measured. Subjects were seen for two visits (V1 and V2), with the visits separated by at least one week; morning (AM) and afternoon (PM) measurements were taken at each visit. A total of four measurements were taken (V1 AM, V1 PM, V2 AM and V2 PM), which all used the same measurement protocol.

Subjects were brought in for an additional visit where fluorescein tear breakup time (FTBUT) was measured by instilling 1 µl of 2 sodium fluorescein dye; three measurements were taken in each eye. Subjects were categorized as having EDE if they had an average FTBUT <5 seconds in one eye.

3.3.3 Statistical Methods

Intraclass Correlation Coefficient (ICC) and Bland-Altman plots were used to assess the test-retest repeatability of intra- and inter-day measurements of OSC rate. The ICC represents the proportion of the total variability that is due to intra-subject variability, which would be attributed to random effects that may occur on repeated measures, with the values ranging from 0 to 1.[125] ICC was classified using the following criteria: > 0.90, very good; 0.70-0.90, good; 0.51-0.70, moderate' 0.31-0.50, moderate; < 0.30, poor.[125] Bland-Altman plots were employed to examine the mean difference between two measurements, with the 95% limits of agreement representing the average difference ± 1.96 standard deviation of the difference.[126]

V1 and V2 intra-day ICC was calculated from the AM and the PM measurements from each visit. Inter-day AM and PM ICC were calculated separately for the two AM and two PM measurements, respectively. Inter-day mean ICC was also calculated using the mean of the AM and PM measurements from each visit (i.e., mean of V1 AM and V1 PM compared to mean of V2 AM and V2 PM). ICC values were assessed for all subjects, and then separately for subjects with and without EDE.

Linear mixed effects model, which accounts for potential within-subject correlations related to repeated OST measurements, was used to compare OSC rate between subjects with and without EDE. Paired t-test was employed to compare OSC

rate between the AM and PM measurements. Analysis for fixed bias was assessed using a one-sample t-test. The results with $p \leq 0.05$ were considered statistically significant for all tests.

3.4 Results

3.4.1 Subject Characteristics

A sample of forty-two subjects (36 females, 6 males) with a mean (SD) age of 23.2 (3.8) years (range: 18-34 years) successfully completed the study and provided data for analysis. The study cohort was composed of 22 Asians and 20 non-Asians. Mean (SD) core-body temperature was 36.6 (0.6) degrees Celsius (range: 32.4-37.3 degrees). Mean (SD) room temperature was 24.8 (0.7) degrees Celsius (range: 22.7-26.2 degree). Mean (SD) room humidity was 47.8 (5.0) percent relative humidity (range: 30.0-58.0 percent).

Nineteen subjects were categorized as having EDE and twenty-three subjects did not have EDE. There was no difference in baseline characteristics between the two groups, except there were more Asians in the EDE group (Table 3-1). Mean OSC rate for subjects with EDE (0.10 ± 0.06^0C/sec) was significantly greater than for subjects without EDE (0.07 ± 0.05^0C/sec) on linear mixed-effects model (p=0.04) (Figure 3-1).

	Non-EDE (n=23)	EDE (n=19)	p-value
Gender	83% Female/17% Male	89% Female/11% Male	n/a
Ethnicity	10 Asians/13 Non-Asians	12 Asians/7 Non-Asians	n/a
Age (SD)	23.1 (4.0)	23.4 (4.0)	0.84
Years of CLW (SD)	9.5 (3.7)	7.8 (4.8)	0.19
Hours of Daily CLW (SD)	11.9 (3.0)	10.4 (4.0)	0.19

Table 3-1 Baseline characteristics for non-EDE and EDE subjects.

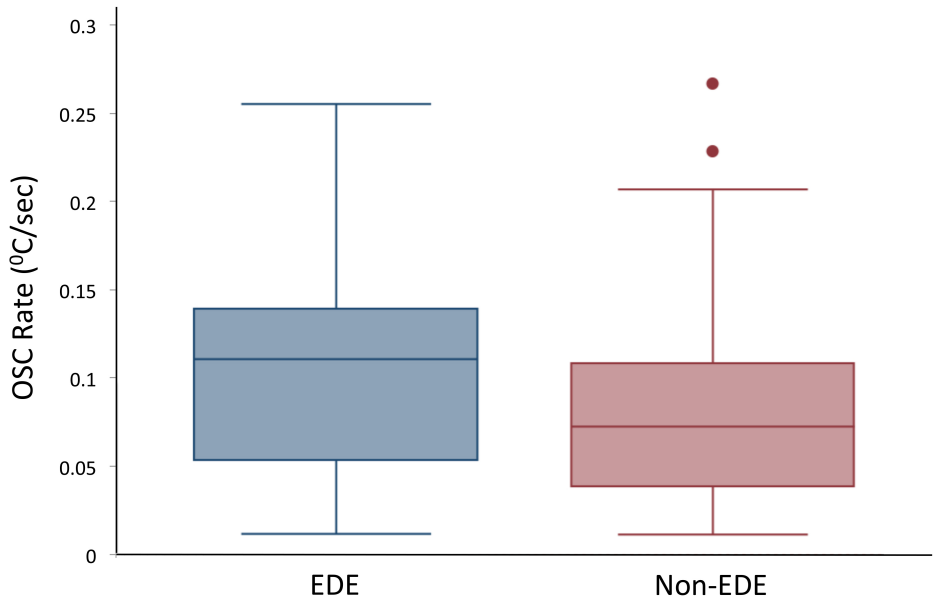

Figure 3-1 Graph box plot showing OSC rate for subjects with and without EDE.

3.4.2 Repeatability (For all subjects)

For all subjects, grand mean OSC rate (SD) was 0.09 (0.06)^{0}C/sec (range: 0.002-0.270^{0}C/sec). There were no significant intra- or inter-day differences in OSC rate (paired t-test; p=0.82 and 0.87, respectively). V1 intra-day ICC was 0.41, V2 intra-day ICC was 0.40, AM inter-day ICC was 0.63, PM inter-day ICC was 0.42 and mean inter-day ICC was 0.70; no fixed bias was noted. Table 3-2 provides a summary of statistical findings for the repeatability of OSC rate measurements in all subjects. Figure 3-2 shows the Bland-Altman plots for all repeatability assessments.

Test	Mean Difference (SD)	p-value (Difference to Zero)	95% Limits of Agreement	Intraclass Correlation
V1 intra-day	-0.002 (0.010)	0.84	-0.13 to 0.13	0.41
V2 intra-day	0.007 (0.008)	0.41	-0.11 to 0.12	0.40
AM inter-day	-0.004 (0.008)	0.58	-0.10 to 0.10	0.63
PM inter-day	0.003 (0.009)	0.72	-0.11 to 0.12	0.42
Mean inter-day	0.003 (0.008)	0.64	-0.07 to 0.07	0.70

Table 3-2 The mean difference, analysis for fixed bias, 95% limits of agreement and intraclass correlation coefficient for all subjects.

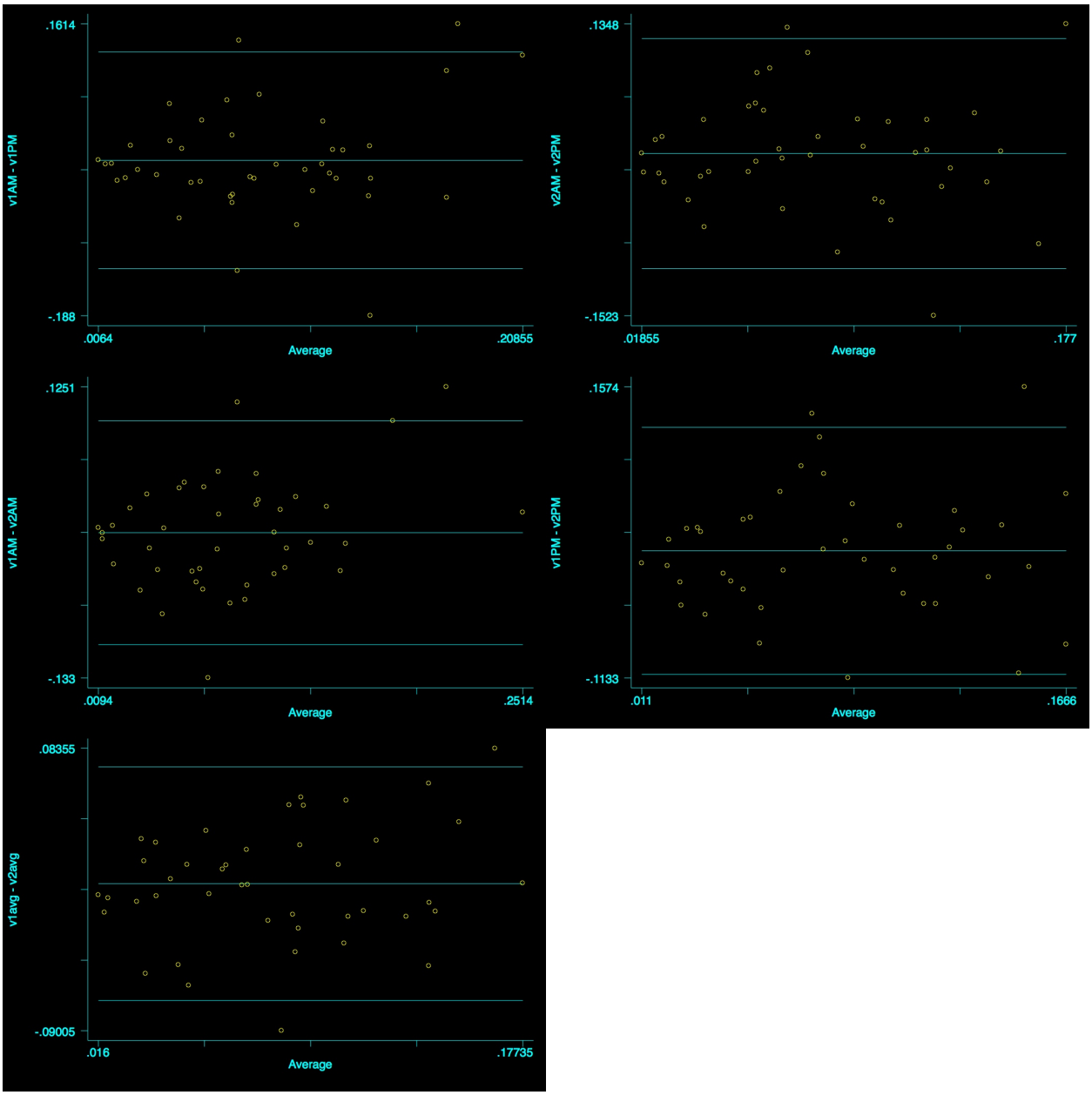

Figure 3-2 Bland-Altman plots for V1 intra-day (Top row, Left), V2 intra-day (Top row, Right), AM inter-day (Middle row, Left), PM inter-day (Middle row, Right) and mean inter-day OSC rate measurements (Bottom row, Left) in all subjects.

3.4.3 Repeatability for non-EDE

For non-EDE subjects, V1 intra-day ICC was 0.49, V2 intra-day ICC was 0.32, AM inter-day ICC was 0.57, PM inter-day ICC was 0.27 and mean inter-day ICC was 0.54; no fixed bias was noted. Table 3-3 provides a summary of statistical findings for the repeatability of OSC rate measurements in non-EDE. Figure 3-3 shows the Bland-Altman plots for all repeatability assessments.

Test	Mean Difference (SD)	p-value (Difference to Zero)	95% Limits of Agreement	Intraclass Correlation
V1 intra-day	-0.007 (0.06)	0.54	-0.12 to 0.11	0.49
V2 intra-day	-0.005 (0.06)	0.67	-0.12 to 0.11	0.32
AM inter-day	-0.005 (0.05)	0.60	-0.10 to 0.09	0.57
PM inter-day	-0.003 (0.07)	0.83	-0.14 to 0.13	0.27
Mean inter-day	-0.004 (0.04)	0.65	-0.09 to 0.08	0.54

Table 3-3 The mean difference, analysis for fixed bias, 95% limits of agreement and intraclass correlation coefficient for non-EDE subjects.

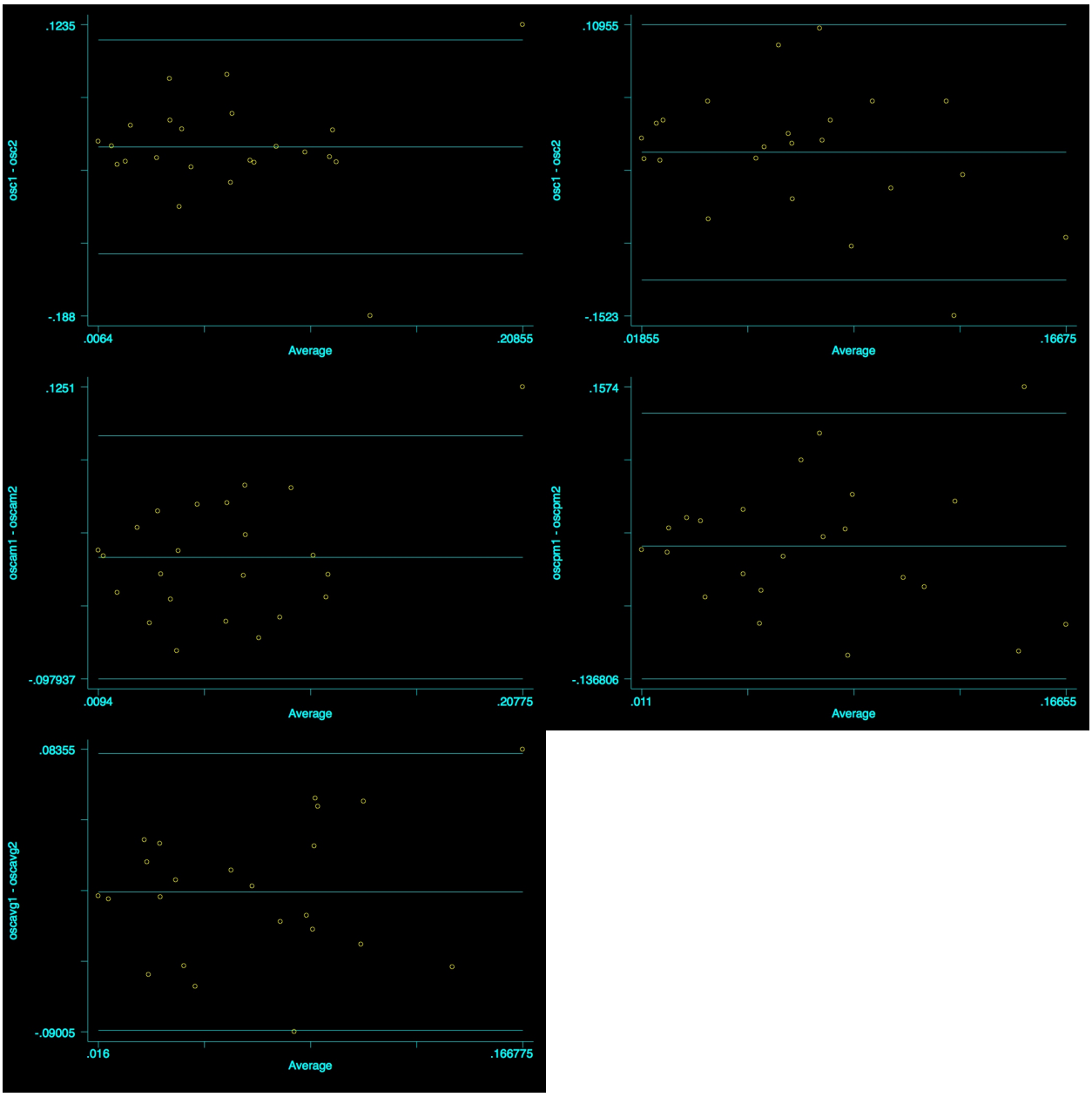

Figure 3-3 Bland-Altman plots for V1 intra-day (Top row, Left), V2 intra-day (Top row, Right), AM inter-day (Middle row, Left), PM inter-day (Middle row, Right) and mean inter-day OSC rate measurements (Bottom row, Left) in non-EDE subjects.

3.4.4 Repeatability for EDE

For EDE subjects, V1 intra-day ICC was 0.24, V2 intra-day ICC was 0.47, AM inter-day ICC was 0.61, PM inter-day ICC was 0.65 and mean inter-day ICC was 0.85; no fixed bias was noted. Table 3-4 provides a summary of statistical findings for the repeatability of OSC rate measurements in EDE subjects. Figure 3-4 shows the Bland-Altman plots for all repeatability assessments.

Test	Mean Difference (SD)	p-value (Difference to Zero)	95% Limits of Agreement	Intraclass Correlation
V1 intra-day	0.005 (0.08)	0.79	-0.15 to 0.16	0.24
V2 intra-day	0.020 (0.05)	0.13	-0.09 to 0.13	0.47
AM inter-day	-0.005 (0.06)	0.73	-0.12 to 0.11	0.61
PM inter-day	0.011 (0.04)	0.29	-0.08 to 0.10	0.65
Mean inter-day	-0.003 (0.03)	0.61	-0.05 to 0.05	0.85

Table 3-4 The mean difference, analysis for fixed bias, 95% limits of agreement and intraclass correlation coefficient for EDE subjects.

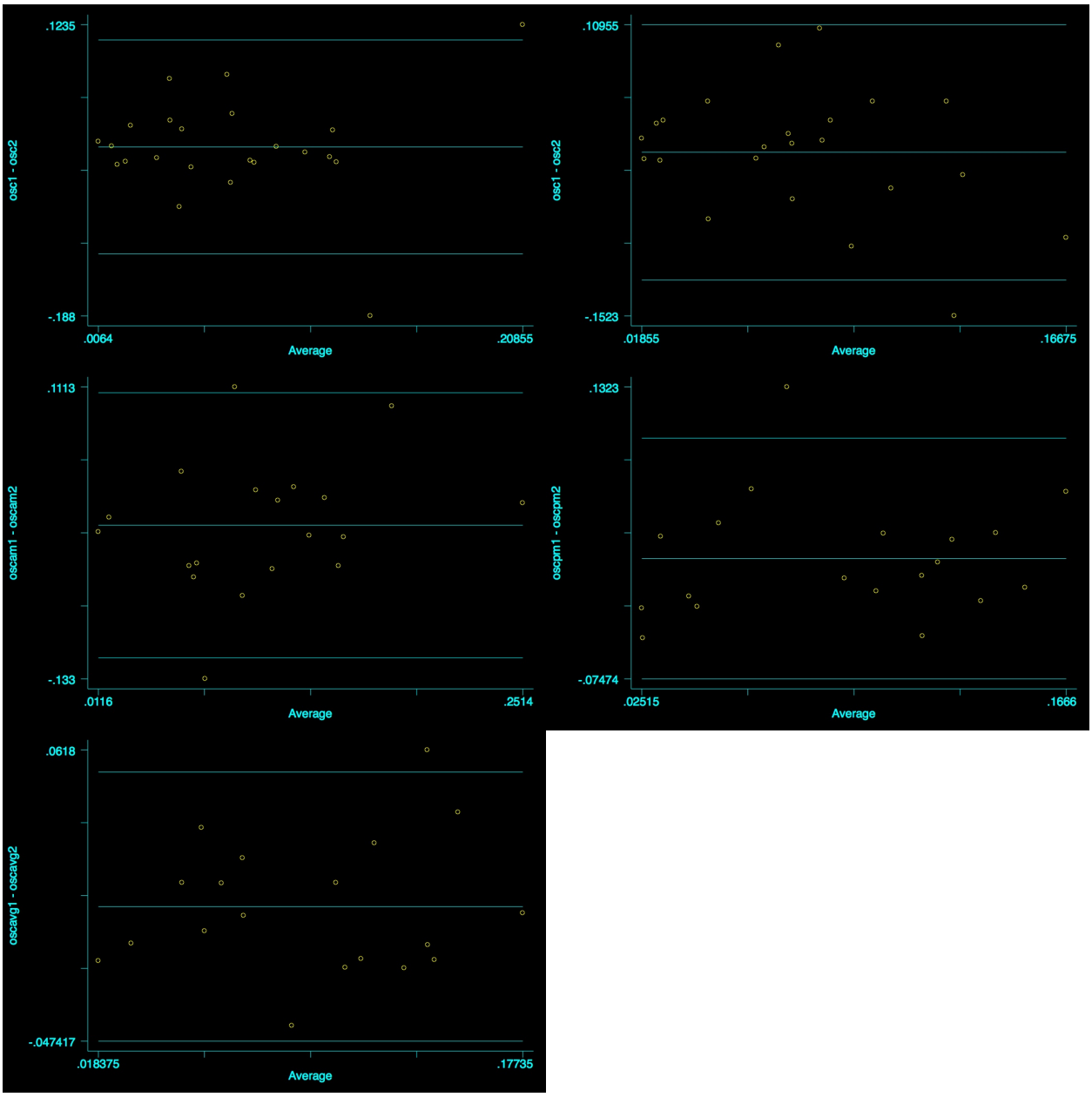

Figure 3-4 Bland-Altman plot for V1 intra-day (Top row, Left), V2 intra-day (Top row, Right), AM inter-day (Middle row, Left), PM inter-day (Middle row, Right) and mean AM and PM inter-day OSC rate measurements (Bottom row, Left) in EDE subjects.

3.5 DISCUSSION

The study found that the measurement repeatability of OSC rate depended on which measurements were used for assessing repeatability. The greatest variability occurred when intra-day measurements were assessed. OSC measurements demonstrated relatively poor inter-day PM repeatability but good inter-day AM

repeatability. This likely reflected the variability in environmental factors and visual demands that subjects encountered before their PM measurements. If true, this would be the first study to demonstrate a diurnal variability of the tear film lipid layer; not accounting for this may be one reason why dry eye tests have such poor repeatability.[127] The best repeatability was achieved when mean AM and PM measurements for each visit were used. The findings suggest the optimal strategy to maximize repeatability for measuring OSC rate, is for it to ideally measured several times in a day, or at the very least, only in the morning.

The study also found that OSC rate was significantly higher in EDE compared than in normal eyes. This is in contrast to a recent study by Abreau et al., which found no difference in OSC rate between normal and EDE, but there were several issues in the study.[128] The study only had 20 subjects total (10 for each group) but most importantly, did not allow the eye to reach a baseline state (close to core body temperature), which likely underestimated the OSC rate. Mathematical models show that heat transfer from the palpebral conjunctiva to the cornea is limited because they are in contact for a short time during a blink.[129,130] As heat from the palpebral conjunctiva is likely not enough to offset the increased cooling in EDE, it is not surprising that EDE tend to have a lower OST after a blink.[128,131] In addition, there is a physiological limit for how low OST can get due to heat conduction from inside the eye.[129,130,132] Therefore, an eye with EDE cannot demonstrate the same range of cooling (the cooling from the initial OST after a blink to the physiological limit) compared to a normal eye, which underestimates the OSC rate. Accordingly, it is important to account for this, which is the reason this study's protocol requires eye closure for two minutes, starting the OST at a baseline state, before measurement.

Interestingly, the study found greater inter-day repeatability of OSC rate in EDE than normal (Figure 3-5). This can likely be explained because when assessing OSC using an IRT, the measurement is influenced by heat loss to the environment (affected by room temperature/humidity) and from evaporation of the tear film. Individuals with EDE have greater repeatability because heat loss from tear film evaporation in EDE is likely significantly greater than from environmental loss, which minimizes a potential source of variability. Therefore, individuals with severe EDE would not only have the highest OSC rate but likely also the greatest measurement repeatability.

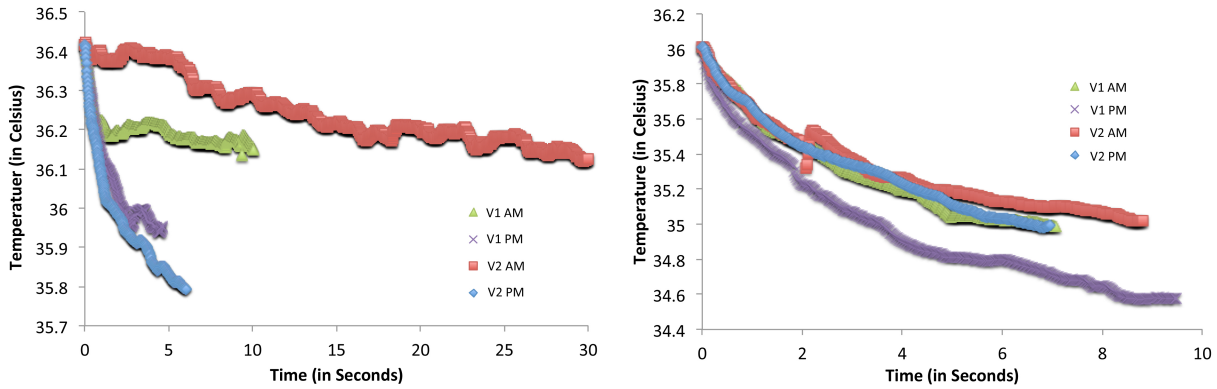

Figure 3-5 The OST profile after a blink showing greater variability in normal (left) than EDE (right) subjects over four measurements.

Additional work is needed to elucidate how best to implement IRT in clinical practice. Due to the relatively poor repeatability in normal eyes, the technique is currently not well suited in diagnosing individuals with EDE. Addressing this weakness would require that we can specifically account for what aspects of the measured OSC rate is due to environmental heat loss and what is due to tear film evaporation.[54,55,57–59] Nevertheless, the high inter-day ICC in EDE, with ICC up to 0.85, demonstrates that IRT may be useful for monitoring EDE. It should be noted that the ICC in our study was higher than ICC that has been reported for other common dry eye test; although our population and diagnosis criteria was different than other studies.[127] The high repeatability of IRT combined with it being non-invasive and ability to detect areas of hyperosmotic stress highlights the clinical potential of IRT.

3.6 CONCLUSIONS

The study found IRT demonstrated poor intra-day repeatability but had fair to good inter-day repeatability. The repeatability of IRT appeared to be influenced by diurnal variability in OSC rate measurements, and should be accounted for. In its current form, IRT would be best implemented in monitoring EDE and more work is warranted to determine the full clinical potential of IRT.

Preface to Chapter 4

Nociceptors are nerves that respond to heat, cold, physical and chemical noxious stimuli. They were a key focus of dry eye research because they appeared to be the primary receptors that were responsible for inducing some of the symptoms associated with EDE. Work in animal *in vivo* models using extracellular single-unit recording of single neurons in the trigeminal ganglion identified the transient receptor potential cation channel, subfamily M, member 8 (TRPM-8) as the receptors likely responsible for the symptoms experienced during EDE.[33,71–74] This was an important identification as TRPM-8 receptors are known for being cold receptors, which at first glance appears to be odd as we are not normally exposed to the cold. Upon closer inspection, TRPM-8 nociceptors are ideal receptors because they are finely attuned to small changes in the OST, primarily to the cooling that occurs on the ocular surface from tear-film evaporation. It was also identified that not only is TRPM-8 nociceptors activated by cooling but also by hyperosmolarity. Interesting, there is a synergistic activation, creating a summation of signaling, of the nerve is observed when both stimuli are present.[33,75] This is important to note when considering the strong evidence to suggest that a localized region of elevated tear film evaporation leads to cooling and hyperosmolarity.[25,26,32,66,76] Therefore, we wanted to obtain a greater understanding of how IRT may potentially be used to quantify the level of irritation on the ocular surface in EDE, with a higher OSC rate associated with greater discomfort.

One issue with measuring discomfort is how subjective and unique it is for each individual. To overcome this issue, we looked for a more objective measure for ocular discomfort. Since individuals are often forced to blink due to discomfort that they experience, we thought that we could assess how long individuals could refrain from blinking as a means to assess the physical manifestation for the discomfort as an indirect measurement for ocular discomfort. We hypothesized that the length of time a subject refrained from blinking could potentially be used to measure an individual's physical response to OSC-induced discomfort (i.e., individuals could refrain from blinking longer if there was minimal OSC), which serves as a protective mechanism for the eye from irritating stimuli.[133]

Chapter 4: Repeated Tear Film Stress Associated with Increased Rate of Ocular Surface Cooling

4.1 ABSTRACT

Purpose: To determine how repeated periods of blink refrainment affect ocular surface cooling (OSC) rates and its potential role in computer vision syndrome (CVS).
Methods: Subjects were acclimated to the exam room for 10 minutes before measurements. They were asked to open their eyes and refrain from blinking for as long as they could, even if they experienced discomfort, while ocular surface temperature (OST) was measured. Each measurement period was termed the maximum inter-blink period (MIBP). Subjects completed ten MIBP with four successive blinks separating each period. OST was measured using an infrared thermographer (FLIR A655sc).
Results: Eighteen subjects (11 females, 7 males) completed the study. On average, subjects had a greater OSC rate (0.12 ± 0.09^0C/s) during the tenth MIBP than in the first MIBP (0.07 ± 0.07^0C/s; paired t-test p=0.02). Grand mean OSC rate was 0.11^0C/s (range: 0.01-0.35^0C/s). Using a linear mixed effects model, we estimated that each additional MIBP was associated with an increase in OSC rate of 0.004^0C/s; over ten periods, this translates to an increase in OSC rate of 0.04^0C/s.
Conclusions: The OSC rate increases with repeated periods of blink refrainment, which suggests that blink refrainment adversely affects the function of the tear film lipid layer, contributing to increased tear evaporation.

4.2 INTRODUCTION

The Digital Age has been marked by increased computer use in everyday life, with its use doubling in the last twenty-five years.[39] In addition, it is no longer limited to the workplace but has increasingly encompassed all aspects of life, with significant time spent on social media, entertainment and communication. As a result, Americans spend an average of 8.5 hours a day in front of a digital device (e.g., computer, smartphone).[40] Unfortunately, prolonged use can lead to symptoms of ocular irritation, redness, dryness, tearing and fatigue, which is known as computer vision syndrome (CVS).[41,134,135] CVS affects 64-90% of individuals who spend at least two hours on the computer, with symptom severity associated with length of use.[44,136] Three million eye exams in the United States were attributed to chief complaints associated with visual or ocular discomfort during computer use. Two billion dollars per year were spent on the diagnosis and treatment of CVS, and sufferers often experience a significant decline in worker productivity.[15,16]

One primary cause for CVS is the tear film change that manifest after individuals spend an extended period on a computer; this is likely attributed to the 50-60% reduction in blink rate when common computer tasks are done (e.g., writing an e-mail or reading the news), due to an increased cognitive load.[41–43] Although blink rate is similarly reduced when reading a book or listening to music, the computer's multi-functionality (e.g., web browsing, working on word documents or spreadsheets,

watching video clips, etc.) allows individuals to spend countless hours on it while only taking minimal breaks.[44]

The portion of the tear-film most sensitive to a decreased blink rate is likely the tear film lipid layer (TFLL), as blinking is required for secretion and spread of meibum over the ocular surface.[36–38] This is an issue because the TFLL plays a major role in inhibiting tear evaporation due to its high local mass transfer resistance, which is theorized to decrease tear evaporation by 75-90%.[21–23] Mathematical models and *in vitro* studies suggest that the TFLL does not lose its evaporation inhibitory properties because it is thinner but as a result of localized areas of TFLL rupture (i.e., absence of TFLL) that form during an inter-blink period.[24,25] Without the TFLL, an area of the ocular surface would experience elevated tear evaporation, leading to tear hyperosmolarity and ocular discomfort.[25–28] Based on this understanding, we theorize that a decreased blink rate will cause the TFLL to have a higher propensity to rupture, leading to greater tear evaporation and ocular discomfort.

Nevertheless, the inability to dynamically measure tear evaporation has prevented investigation to confirm this theory.[6,49,50] Recently, our group determined that measuring ocular surface temperature (OST) using infrared thermography (IRT) indirectly measures tear evaporation rate, with greater ocular surface cooling (OSC) associated with higher tear evaporation.[122] When tear evaporation occurs, the phase change from liquid to gas is associated with heat transfer to the surrounding environment, causing a cooling of the ocular surface.[55–59] It was also determined in the study that localized areas of increased OSC represented regions of TFLL rupture.[122] If CVS has an evaporative etiology, IRT may be an ideal tool to gain an insight on CVS because it is non-invasive, allows for visualization of localized TFLL rupture, and provides a dynamic assessment of tear evaporation. The purpose of this study was to use IRT to determine if a decreased blink rate alters the tear evaporation rate and understand how this contributes to symptoms of CVS.

4.3 METHODS

4.3.1 Subjects

Subjects were recruited from the University of California, Berkeley and the surrounding community. Subjects taking systemic or ocular medication, or with a history of ocular disease or surgery were excluded from the study. No exclusion was made based on contact lens wearing status, but subjects were asked to discontinue contact lens wear for at least 24 hours before the appointment. Subjects were instructed to refrain from using any eye makeup or eye drops on the day of the visit. Informed consent, with a complete description of the goals, risks, benefits, and procedures of the study, was obtained from all participants. This study observed the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley Committee for Protection of Human Subjects.

4.3.2 Instrumentation and Procedures

Ocular surface temperature was measured using a FLIR A655sc (FLIR Systems, Inc., Wilsonville, OR, USA) uncooled microbolometer IRT, which has a 640x480 video resolution, 17 µm pixel size, and 0.1°C thermal sensitivity. The IRT, mounted on a tripod, was placed eight to ten inches from the eye, focused on the ocular surface and aligned approximately perpendicular to geometric center of the cornea. FLIR+ Tools software (FLIR Systems, Inc., Wilsonville, OR, USA) was used by an experienced observer (WL) to specify a user-defined region of interest corresponding to the cornea in infrared recordings. The region represented 4,000–7,000 measurement points (accounting for anatomical variation in palpebral aperture size), with mean value of points interpreted as mean OST and used to calculate a linear OSC rate, degree Celisus per second (^{0}C/sec).[55] Due to the correlation between OST and core body temperature, subject core temperature was measured using an Exergen temporal artery thermometer (Exergen Corporation, Watertown, MA, USA).[124] Room temperature and humidity were measured using a digital thermometer (General Tools & Instruments, Secaucus, NJ, USA).

Subjects were asked to complete an Ocular Surface Disease Index (OSDI) questionnaire prior to measurements. An anterior segment examination under white light was performed to ensure there was no active or pre-existing ocular pathology (e.g., infiltrates, excessive corneal epithelial irritation). Subjects were taken to another room where they were acclimated to the ambient environment for a minimal of ten minutes before testing.[64] TFLL thickness and a blink count was assessed using LipiView® interferometry (TearScience Inc, Morrisville, NC, USA). Non-invasive tear break-up time (NITBUT) was measured twice using Medmont E-300 corneal topographer (Medmont, Melbourne, Australia), with 30 seconds elapsed between each measurement.

Subjects were placed in a slit lamp head- and chinrest assembly, which minimized head movement during OST recording. They were then asked to open their eyes and refrain from blinking or moving their eyes for as long as possible, even if they experienced discomfort, while OST was measured; this was termed the Maximum Inter-Blink Period (MIBP). Ten successive MIBPs were done in the right eye, with each period separated by four rapid blinks (Figure 4-1). Shortly after ten MIBPs, LipiView® and NITBUT measurements were repeated. The time length for MIBP was determined based on reviewing recordings after the study.

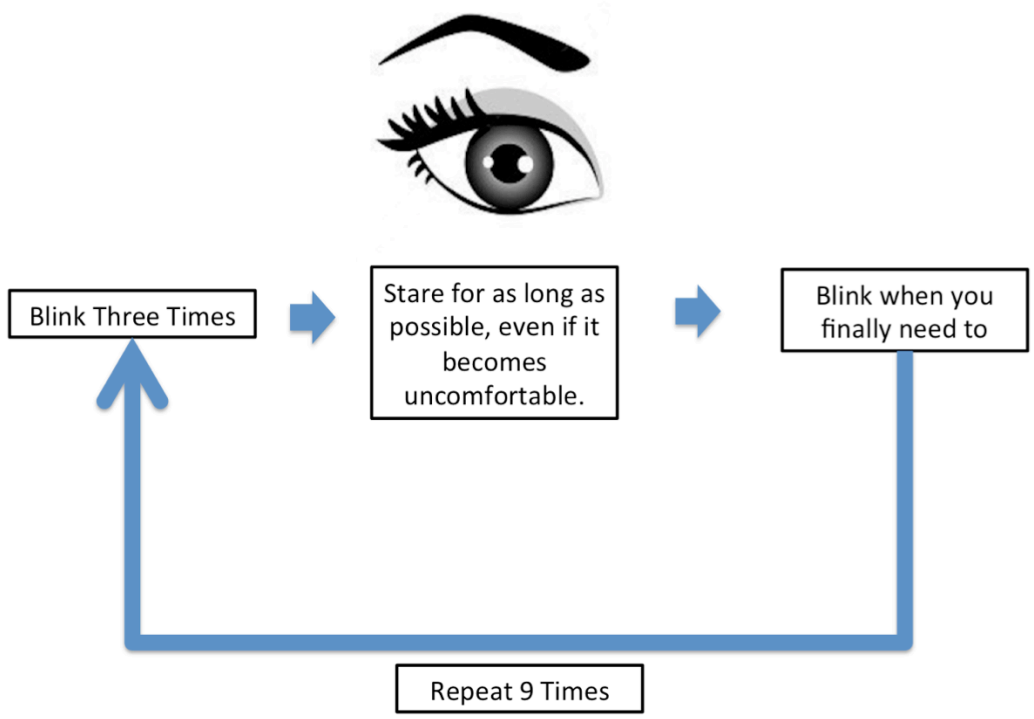

Figure 4-1 Visual instructions provided to subject

4.3.3 Statistical Methods

As the time length for MIBP length was variable (i.e., each person refrained from blinking for different lengths of time), analysis was done that accounted for OSC rate during an entire MIBP and for the first three seconds of a MIBP, which was done to standardize time. Five MIBPs (in four different subjects) had time lengths that were shorter than three seconds; in these cases, we calculated the OSC rate based on the maximum time that subjects could keep their eyes open, which ranged from 1.1 to 2.8 secs.

During preliminary exploratory analysis (not shown), we found that OSC rate from the first three seconds of a MIBP was more appropriate for statistical modeling than from an entire MIBP length. This was likely because the cooling profile observed over an entire MIBP was an exponential curve and not a linear function; in contrast, the first three seconds demonstrated good linear approximation (R^2=0.81), while still representing when most of the cooling occurred (64% of total cooling). In addition, accounting for only the first three seconds of a MIBP potentially limited external factors such as social desirability bias, pain sensitivity and afferent pathways that could influence how long an individual could refrain from blinking.[85,137–139] Therefore, the analysis will focus on OSC rate from the first three seconds of a MIBP and we will refer it as “OSC rate” for the remainder of the manuscript.

Paired t-test was employed to compare OSC rate for the first versus tenth MIBP, and to compare baseline NITBUT and LipiView® measurements with those after ten MIBPs. After a thorough exploratory and descriptive analysis, linear mixed effects

models were used in order to account for potential within-subject correlations related to repeated OST measurements. Separate linear mixed effects models were created to determine (1) what factors were associated with greater OSC rate and (2) if OSC rate influenced MIBP length (i.e., how long they could refrain from blinking). In the first linear mixed effects model, OSC rate was transformed to a natural logarithmic scale (ln[OSC]) to approximate normality for analysis, and in the second model, MIBP length was transformed to a natural logarithmic scale (ln[MIBP]) to approximate normality for analysis. The results with $p \leq 0.05$ were considered statistically significant for all tests.

4.4 RESULTS

4.4.1 Subject Characteristics

A sample of twenty-one subjects was initially recruited for the study. Three subjects were unable to complete the study as they exhibited excessive eye movements during OST recording that contributed to poor data quality. A total of eighteen subjects (11 females, 7 males) with a mean (SD) age of 23.1 (3.5) years (range: 18-32 years) successfully completed the study and provided data for analysis. The study cohort was composed of eight contact lens wearers and ten non-contact lens wearers; eleven subjects were Asian and seven subjects were non-Asian. Mean (SD) OSDI score was 7.6 (9.7) with a range of 0-35.4. Mean (SD) core-body temperature was 36.9 (0.4) degrees Celsius (range: 35.5-37.4 degrees). Mean (SD) room temperature was 22.1 (0.4) degrees Celsius (range: 21.4-23.0 degree). Mean (SD) room humidity was 48.1 (9.4) percent relative humidity (range: 34.0-59.0 percent). Mean (SD) blink count was 6 (3) blinks (range: 2-13 blinks)

Overall, there were 180 MIBPs available for analysis (ten per subject; each data point consisted of an OSC rate and MIBP length). Grand mean OSC rate (SD) was 0.11 (0.08) ^{0}C/sec (range: 0.01-0.35^{0}C/sec). Mean MIBP length (SD) was 17.5 (15.8) secs (range: 1.1-90.5 secs). No difference was found between baseline and after ten MIBPs for NITBUT (19.4 secs vs. 20.0 secs, respectively; paired t-test, p=0.91) and for TFLL thickness (60.9 nm vs. 66.1 nm, respectively; paired t-test, p=0.40).

4.4.2 Effects of Blink Refrainment on OSC rate

A review of infrared recordings showed that in a majority of subjects, areas of OSC increased with each subsequent MIBP and that they appeared to originate in the same area (Figure 4-2). On average, subjects had a significantly greater OSC rate during the tenth MIBP (0.12±0.09^{0}C/sec) than in the first MIBP (0.07 ±0.07^{0}C/sec), paired t-test, p=0.02 (Figure 4-3). Fourteen out of the eighteen subjects had a greater OSC rate at the tenth MIBP than the first MIBP. In the four exceptions, three cases had greater OSC rate on the first MIBP and one case had equal OSC rate on the first and tenth MIBP.

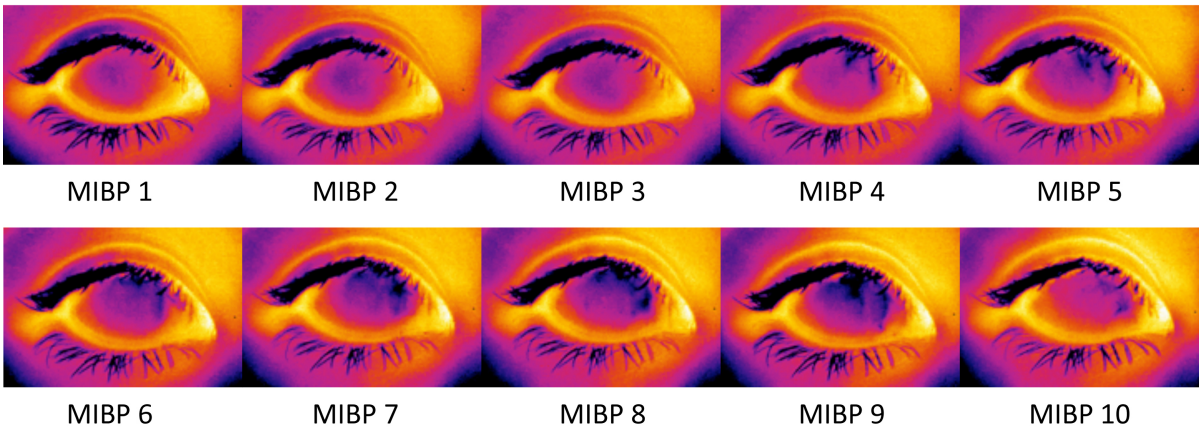

Figure 4-2 Screenshot of infrared recording from Subject TL, shortly before a blink, over ten MIBPs. There is increased OSC (darker areas) after each MIBP, except in the tenth MIBP.

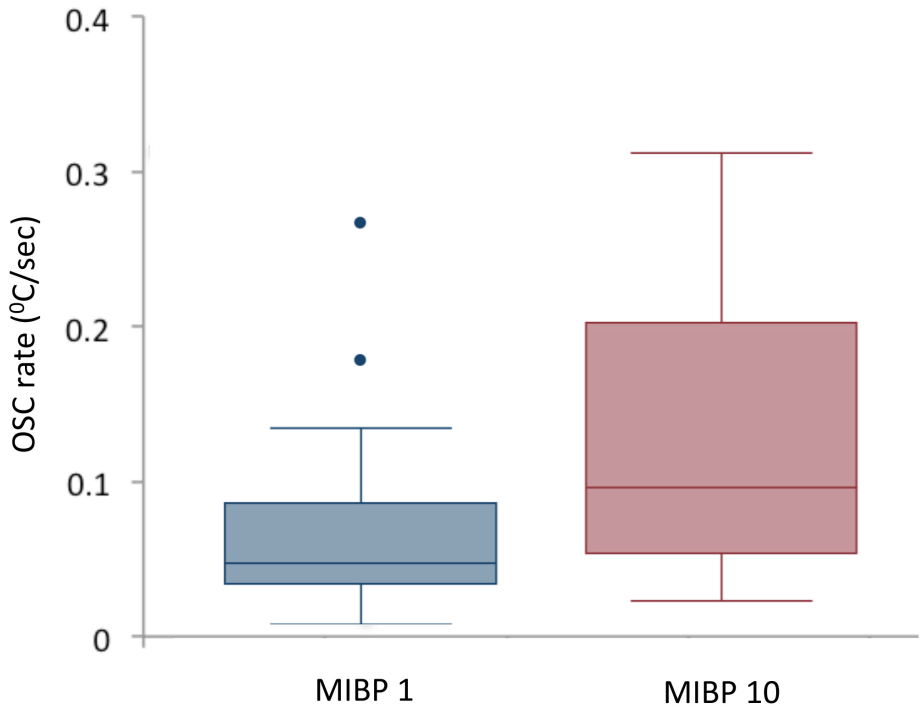

Figure 4-3 Box plot comparing mean OSC rate between the first and tenth MIBP.

Using a linear mixed effects model to account for repeated measures, OSC rate was found to be positively associated with the number of MIBPs done and blinks measured with the LipiView at baseline ($p<0.001$ and $p=0.003$, respectively). In the model, it is estimated that each subsequent MIBP was associated with an increase in OSC rate of 0.004^0C/sec; over ten MIBPs, this translated to an increase in OSC rate of 0.04^0C/sec. Each additional blink measured at baseline was associated with a

0.01^0C/sec increase in OSC rate. No association was seen with TFLL thickness, NITBUT and OSDI.

4.4.3 OSC rate Influences Time-Length of the MIBP

Examining the recordings, there appeared to be a trend where greater OSC rate was associated with shorter MIBP (Figure 4-4, 4-5). Figure 4-6 shows a scatter plot of all 180 MIBP, examining OSC rate and a natural log-transformed MIBP length. A linear mixed effects model with a natural log-transformed MIBP length showed that increased OSC rate was significantly related to a shorter MIBP ($p<0.001$). In the model, it is estimated that an individual would be able to refrain from blinking for an additional 16.1 seconds if they had the minimum OSC rate found in the study (0.01^0C/secs) when compared to someone with the maximum OSC rate (0.35^0C/secs). No association was seen with TFLL thickness, blink count, NITBUT and OSDI.

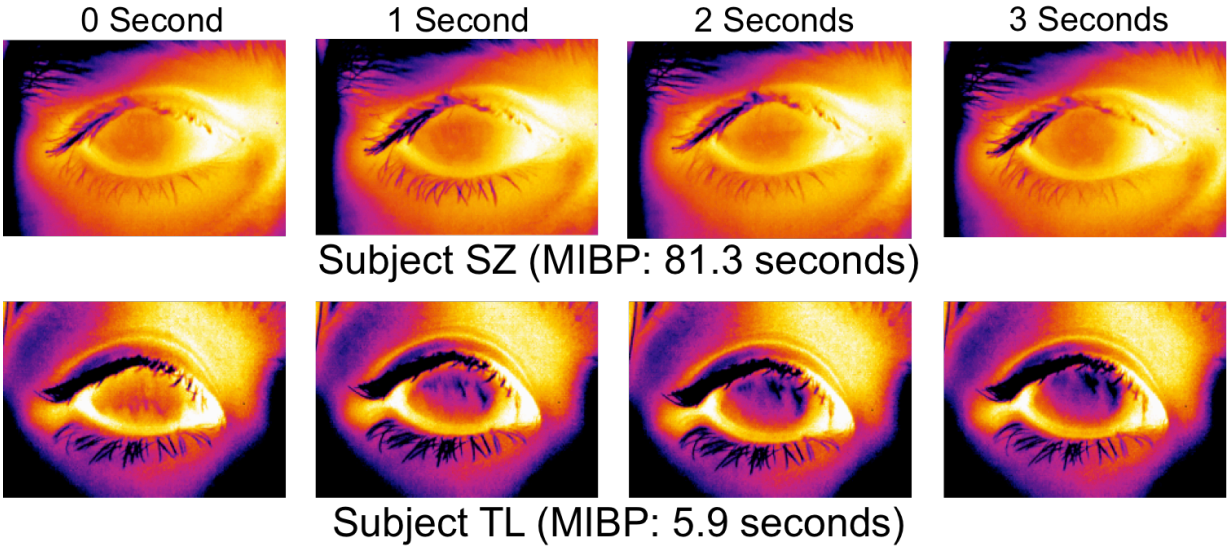

Figure 4-4 Subject SZ with a MIBP of 81.3 seconds was observed to have minimal temperature changes, indicated by less color changes, compared to Subject TL with a MIBP of 5.9 seconds.

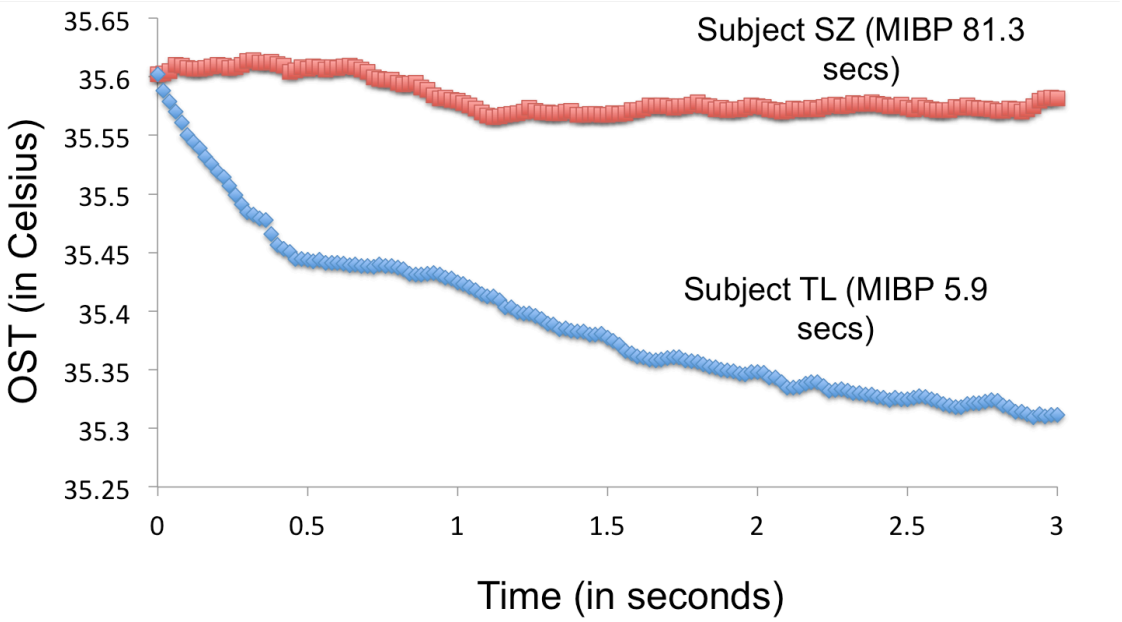

Figure 4-5 Initial five seconds of ocular surface temperature profile of Subjects SZ and TL, from Figure 2, are shown. The profile shows a steeper OSC rate in Subject TL than in Subject SZ.

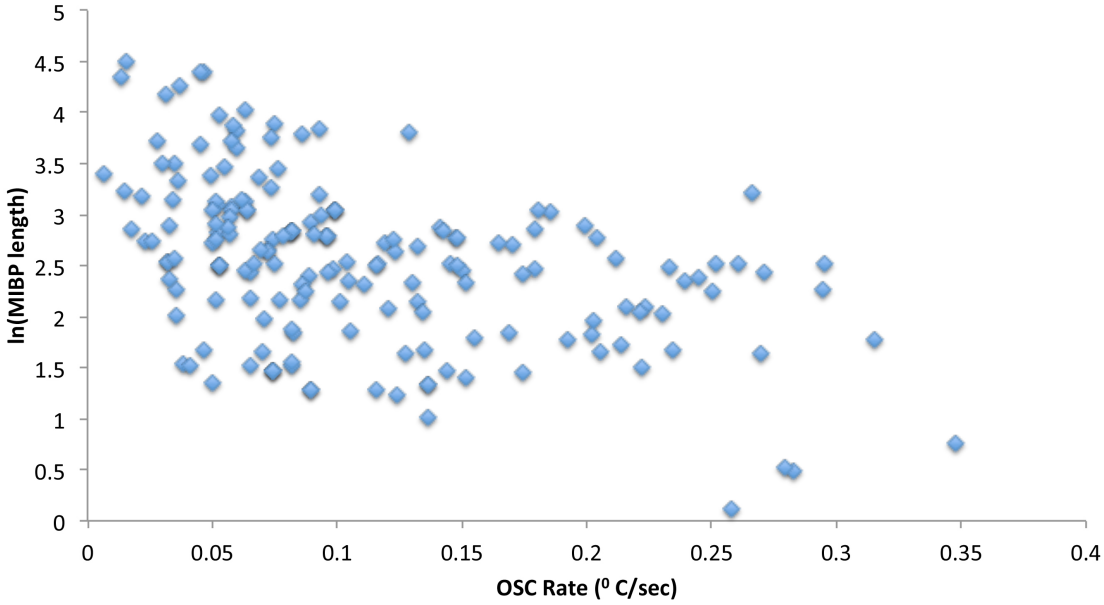

Figure 4-6 Scatterplot examining OSC rate and natural-log transformed MIBP time length for all 180 MIBPs.

4.5 DISCUSSION

The study found that having subjects refrain from blinking contributed to a greater OSC rate (indirectly representing higher evaporation); the effects were found to be cumulative, with OSC rate increasing with each subsequent MIBP. In the three cases where OSC rate was greater in the first than tenth MIBP, there was only a marginal difference in one subject, and the other two subjects were observed to have significant reflex tearing. The heat from reflex tears, which is close core body temperature, likely offset the increased cooling on the ocular surface (Figure 4-7). In the case where OSC rate was equal between the first and tenth MIBP, there may been enough tear film stress the subject to disrupt the TFLL, as the subject was characterized as having high tear film stability at baseline (NITBUT >30 seconds). The increased tear evaporation observed is likely due to the role that a decreased blink rate has on the TFLL, making it more prone to rupture, as a decreased blink rate potentially causes: (1) less meibum to be secreted from meibomian glands (leading to thinner TFLL), (2) an alteration in the TFLL composition, and/or (3) the prevention of an optimal organization of the TFLL.

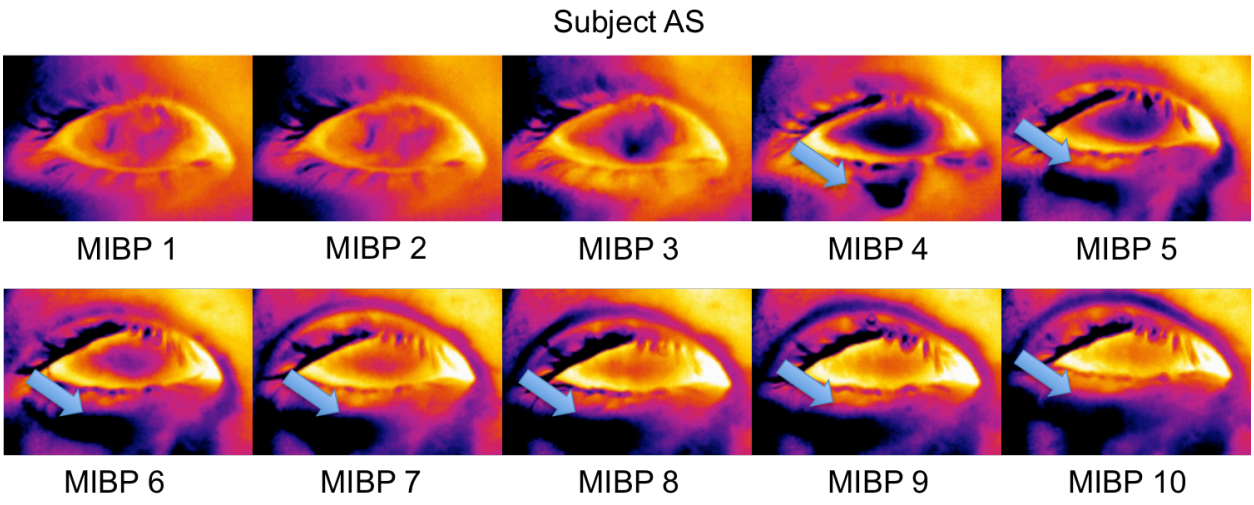

Figure 4-7 Subject AS showing significant reflex tearing after the 4th MIBP and continues to worsen over subsequent MIBPs.

Since the muscles involved in a blink are also involved in meibum secretion, a decreased blink rate likely caused less meibum secretion, leading to a thinner TFLL.[36,140–142] Interestingly, in our study, TFLL thickness slightly increased after ten MIBPs, a 6 nm increase was observed, although the difference was not statistically significant. This may be due to instrument variance of the LipiView® or that ocular discomfort experienced after multiple MIBPs, led to stronger blinks that contributed to increased meibum secretion, off-setting the initial decline in secretion. This is supported by Korb's study, which found that three strong blinks could increase the TFLL thickness by 19-33 nm (assessed using the Tearscope®).[143] There may also be a change in meibum composition, as the force generated by a stronger blink may cause lipids to come from undifferentiated (immature) meibocyte on the basal layer of acinar glands, instead of solely from differentiated (mature) meibocytes.[36] Microscopy has shown that there is a significant difference in cellular composition between immature and mature

meibocytes; the maturation process may make lipids better at inhibiting evaporation.[36,144] This has been offered as one reason for why *in vitro* studies using extracted meibum, obtained by applying significant force on eyelids, have been unable to recreate the TFLL's ability to inhibit evaporation as observed *in vivo*.[23] Therefore, lipids from immature meibocytes could make the TFLL more prone to rupture during an inter-blink period.

Nevertheless, these two explanations fail to account for what was observed in some subjects. As noted in Figure 4-8, upon repeated MIBPs, the area of OSC developed in the same region, maintained a similar geometric pattern, and grew with each subsequent MIBP. This was unlikely a continual lack of TFLL in those areas, as TFLL fluidity would allow four blinks to fill in an area of rupture.[145,146] In addition, it could not be explained by a change in TFLL composition, as it globally affects the tear film, not just in the same localized area. A third possibility is that there is an organization of lipids in the TFLL, as suggested by computational models and *in vitro* studies.[147,148] An optimal organization of the lipids could theoretically inhibit excessive tear-film evaporation by decreasing the proclivity for the TFLL to rupture during an inter-blink period. In the study, the MIBP likely induced tear film stress that disrupted the organization in an area of the ocular surface; without the necessary time to reorganize, the same area would be vulnerable to more rapid and extensive rupture on subsequent MIBPs. However, as significant aspects of the TFLL are still unknown, more work is needed to conclusively determine the factors that contributed to our observations and findings.

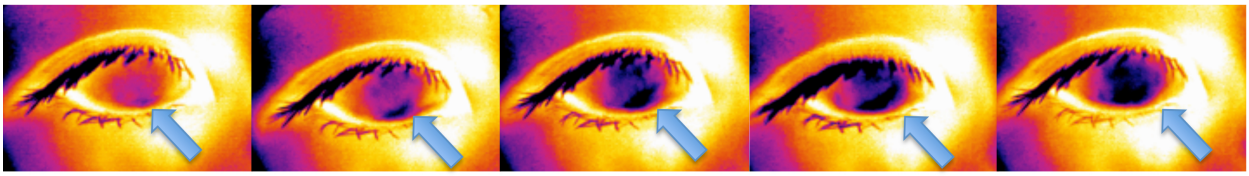

Figure 4-8 Screenshot of infrared recording from Subject EH, shortly before a blink, from the fourth to the eighth MIBPs. The area of OSC (arrow) is developing from the same region and is growing larger over subsequent MIBPs.

This study provides a possible explanation for why CVS sufferers experience increasing discomfort during the course of a day while in front of a computer. Animal *in vivo* models have identified the transient receptor potential cation channel, subfamily M, member 8 (TRPM-8) as the nociceptors likely responsible symptoms experienced in dry eye.[33,71–74] The TRPM-8 receptors are activated by hyperosmolarity and cooling; a synergistic activation of the nerve is observed when both stimuli are applied.[33,75] An area of TFLL rupture creates a region of elevated tear evaporation, forming a localized spot of cooling and hyperosmolarity.[25,26,32,66,76] Therefore, if a decreased blink rate causes a greater tendency for TFLL rupture, it is assumed that nociceptors would be increasingly activated and create greater discomfort. This is partially supported by our study, which found that a greater OSC rate was associated with a shorter MIBP length. The greater OSC activated more corneal nociceptors, creating ocular discomfort; the discomfort served as an impetus for subjects to blink more quickly.

A potential issue with this study is that asking subjects to refrain from blinking is not a natural phenomenon. Nevertheless, this experimental model was chosen because we concluded that this was the best method to mimic, within a short period of time, what occurs to the TFLL after hours of a decreased blink rate in front of a computer. Another issue is that the study appears to show a contradiction, as a lower blink count at baseline was associated with a lower OSC rate, while a decreased blink rate was correlated with greater OSC rate. Subjects with a low blink count may have a TFLL that is better at inhibiting tear evaporation requiring them to blink less. Yet, if the subject had a decreased blink rate, relative to their baseline blink rate, there would likely be a long-term effect on the TFLL's ability to prevent rupture. Finally, as NITBUT represents tear film stability, it may seem surprising that there was no difference in NITBUT values between baseline and after ten MIBPs. This is was likely because the subjects experienced reflex tearing, which was observed in several subjects, from the irritation induced by having them refrain from blinking; reflex tearing is known to artificially increase NITBUT.[47]

This study provides insight on possible tear-film changes that contributes to CVS and Figure 4-9 shows a possible causal pathway for CVS. It is assumed that in the morning, shortly after waking up, the TFLL is optimized for inhibiting tear evaporation. When individuals spend time on digital devices, there is often a 50-60% decrease in blink rate. Over an extended time, a decreased blink rate compromises the TFLL, increasing the propensity and area of TFLL rupture, as suggested by our study. As a result, increased tear evaporation occurs, leading to activation of nociceptors and inducing ocular discomfort. If this pathway is accurate, prevention of CVS will be dependent on developing ergonomic and treatment options to disrupt it. Initial steps to remedy this problem have been made as efforts have been put forward to educate and encourage computer users to take more breaks. New technologies have also been developed to remind individuals to blink more often but the growing use of computers and smartphones may require that additional steps be taken.

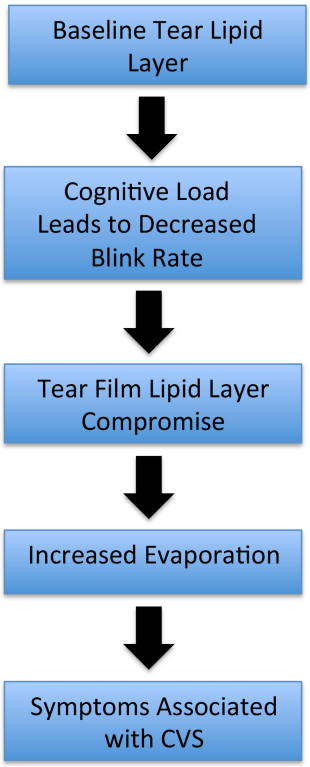

Figure 4-9 Proposed causal pathway for CVS symptoms due to tear film changes.

4.6 CONCLUSION

The OSC rate increases with repeated periods of blink refrainment, which suggests that a similar rate increase occurs after extended periods of near work. This may partially explain the increased ocular discomfort noted by many patients after prolonged near work, during which the blink rate is reduced, and TFLL rupture and tear evaporation are increased.

Preface to Chapter 5

Even if IRT measures ocular surface irritation, it may not translate to understanding how it is ultimately perceived, as pain sensitivity is highly individualized.[80–82] This can be seen from Figure P5-1, which is the results of the study described in Chapter 3 of the thesis. The large inter-subject variability in MIBP observed in the study may be due to differences in pain sensitivity among different individuals (Figure P5-1). We felt further investigation was warranted to confirm the relationship between ocular TFS, as reflected by the MIBP length, and pain sensitivity.

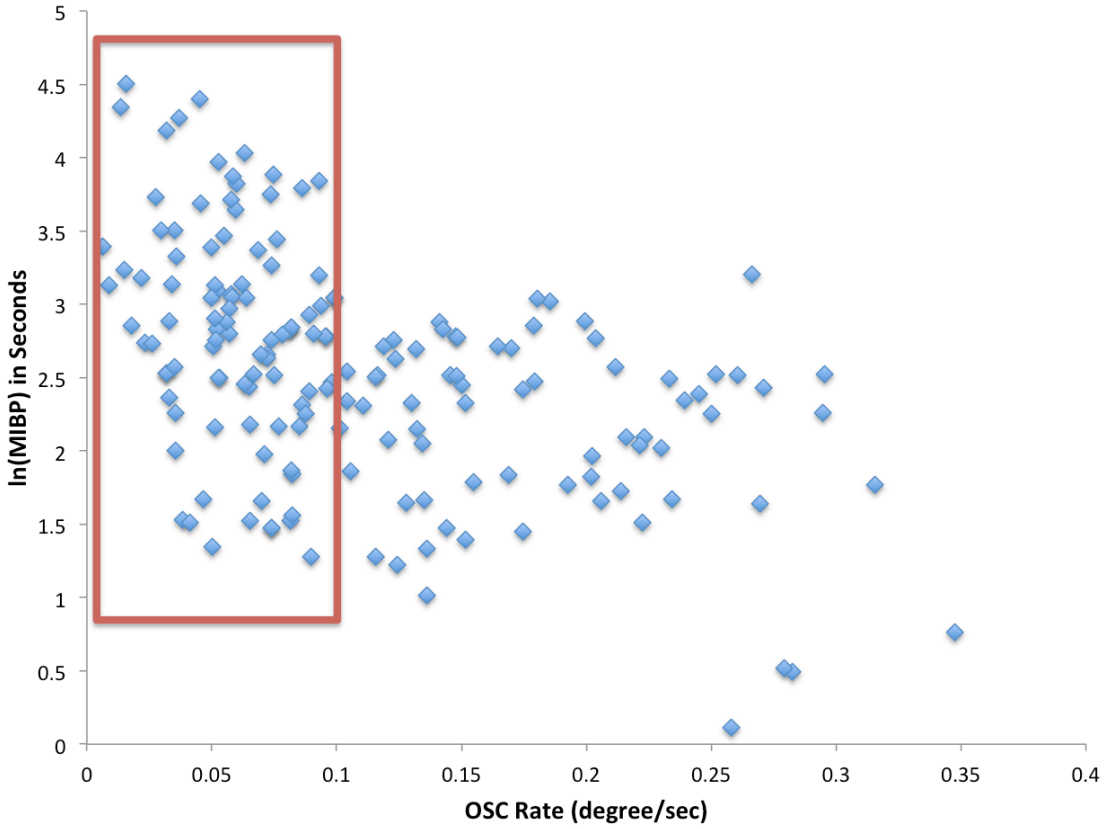

Figure P5-1 OSC rate and ln(MIBP) for 180 measurement periods (18 subjects each with 10 measurement points). The red bracket shows the large inter-subject variability in MIBP observed in the study.

Figure P5-1 represents the significant discrepancy between clinical signs and patient symptoms of ocular discomfort that is often noted [29,77,149,150]. A typical example of this discrepancy can be seen in regards to dry eyes, as it is not uncommon for patients to report dry eye symptoms but lack clinical signs or conversely, present with signs but be asymptomatic, and studies have found a lack of association between signs and symptoms in dry eye disease [10,13,29,46,77]. The lack of progress in understanding the relationship between signs and symptoms of ocular discomfort may be due to the failure in recognizing that the level of ocular discomfort experienced is not merely defined by

the extent of ocular surface disruption (e.g., by contact lens or excessive tear evaporation) but also by how it is perceived [29,77–79].

It would not be unexpected if an identical ocular irritant, applied to a group of individuals, led to a diverse range in the level of discomfort reported.[80,83,84] Therefore, a link between OSC rate and EDE symptoms may not be found unless pain sensitivity is taken into account. Only limited work has been done on pain sensitivity and ocular discomfort, as experimentally measuring pain sensitivity is difficult and inducing pain on subjects can have a profound impact on how they perceive an irritant or answer questionnaires.[151–153] This led us to find out if the PSQ overcomes these challenges and could be an effective tool in clinical studies.

Chapter 5: Understanding ocular discomfort and dryness using the Pain Sensitivity Questionnaire

Note: This chapter will soon to be published under the same title in PLOS One. The co-authors were Andrew Graham and Meng C. Lin; permission to include this material in the dissertation was received from both of them.

5.1 ABSTRACT

Purpose: To utilize the Pain Sensitivity Questionnaire (PSQ) to assess the influence of pain sensitivity on perceptions of ocular discomfort and dryness.
Methods: Subjects completed a battery of questionnaires, including history of ocular and general health, contact lens wear history, the Ocular Surface Disease Index (OSDI) questionnaire, visual analog scale (VAS) 100-point rating scales to assess severity and frequency of average and end of day (EOD) discomfort and dryness, and the PSQ to assess pain sensitivity level. Masked subjects were then instructed to wear one inverted and one normally oriented soft contact lens contralaterally for 30 minutes to induce an inter-eye difference in comfort and dryness sensations. Subjects rated comfort and dryness in each eye on VAS every 5 minutes during contact lens wear. A slit lamp examination was performed to evaluate ocular surface health and to assess contact lens fit.
Results: One hundred and fifty-three subjects (111 females, 42 males) completed the study. In separate models, a higher PSQ score was significantly associated with higher OSDI score (p=0.002), lower average and EOD comfort (p=0.005 and 0.001, respectively), and greater EOD dryness (p=0.04). The minimum (0.14) and maximum (7.14) PSQ scores observed in our subject cohort (i.e., from the subjects who were the least and most sensitive to pain, respectively) corresponded to an estimated difference of 11 points on the OSDI, 20 points on the VAS scale for average comfort, 31 points for EOD comfort and 17 points for EOD dryness. In a mixed effects model, a higher PSQ score was significantly associated with a greater inter-eye difference in comfort (p=0.013) and dryness (p=0.010) during CL wear.
Conclusions: Pain sensitivity influences perceptions of ocular discomfort and dryness, and should be taken into account when evaluating subjective assessments of these symptoms.

5.2 INTRODUCTION

Due to limitations with diagnostic tests that assess the ocular surface, clinicians often rely on subjective questionnaires to assess and monitor ocular discomfort [154]. Despite such a significant reliance, there has been limited investigation into the factors that influence inter-subject differences in ocular discomfort reported. In response to an identical stimulus to discomfort, individuals can differ greatly in how they perceive it and report it on a questionnaire, [80,83–86] with people who are more sensitive to pain or discomfort rating the sensation more extremely than would a less sensitive person.

Therefore, an instrument that provides some insight into how individuals perceive ocular discomfort could be of benefit in interpreting patient symptomology and influencing treatment decisions.

A validated instrument that measures the level of sensitivity to discomfort could also be useful in examining the often noted discrepancy between clinical signs and patient symptoms of [29,77,149,150]. A typical example of this discrepancy can be seen in regards to dry eyes, as it is not uncommon for patients to report dry eye symptoms but lack clinical signs or conversely, present with signs but be asymptomatic, and many studies have found a lack of association between signs and symptoms in dry eye disease [10,13,29,46,77]. Another example is found in patients with CL discomfort. Although studies have identified a number of factors that are associated with greater discomfort during CL wear (e.g., Asian ethnicity, inferior corneal staining, excessive lens movement, CL surface wettability), there is still significant uncertainty regarding the pathophysiology of CL discomfort [149,150,155–158]. The lack of progress in understanding the relationship between signs and symptoms of ocular discomfort may be due to the failure in recognizing that the level of ocular discomfort experienced is not determined solely by the extent of ocular surface disruption but also by how it is perceived [29,77–79].

This is unsurprising, as the perception of ocular discomfort is based on the following neural pathway: (1) the signal (e.g., triggered by an irritant) originates on the ocular surface, (2) is transmitted to the brainstem, (3) then relayed to the limbic system, and (4) finally conveyed to the cerebrum.[77,95] At each step, the signal (and ultimately the perception of ocular discomfort) can either be upregulated or downregulated by nociceptive processing in the brainstem, emotional state in the limbic system, memories of pain in the parietal lobe of the cerebrum and the level of attention given to pain in the frontal lobe of the cerebrum, which are all influenced by a complex interaction of factors.[81,82,96–98]

This is a similar issue to that which pain researchers have faced in attempting to explain why identical injuries can lead to a diverse range of reported pain or discomfort [80,83–86]. An insight into this issue was gained with the recognition that the cognitive modulation of pain or discomfort is highly individualized. This biopsychosocial pain model, which as Green explains, states that “pain is ultimately sculpted by complex and dynamic interactions among biological, psychological and sociocultural processes,”[80,82,85,86,88–94] suggests that pain sensitivity, defined as how individuals rate painful stimuli, is the most important metric in understanding individual pain perception.[92,99,100] In addition, pain sensitivity has been linked with the level of analgesic use after surgery, the risk of developing chronic pain, and how successful a medical procedure is perceived to be. [89,100–103] In the literature, pain sensitivity has been experimentally measured by determining the level of cold, heat or pressure stimuli a patient could withstand before considering it to be painful [92,99]. In such tests, an individual with higher pain sensitivity would notice pain at a lower stimulus level. The potential of using pain sensitivity to understand ocular discomfort was demonstrated by Vehof et al., but the logistical difficulties of experimentally measuring pain sensitivity have prevented it from being widely studied, as the measurements are time-intensive, expensive, depend on specially trained staff and require inducing pain in healthy subjects [92,99,159].

The Pain Sensitivity Questionnaire (PSQ), which was developed by Ruscheweyh et al., may overcome some of these challenges [92]. The PSQ is a self-rating instrument, taking three to five minutes to complete, that asks respondents to imagine themselves in painful situations that are commonly experienced, and to rate the pain they feel they would experience (Figure 5-1). The questionnaire is simple, requiring no equipment or extensive training, inducing no anxiety in subjects or patients at the prospect of an imminent “pain test”, and being quick to complete even with large numbers of research subjects. The PSQ provides a score that rates pain sensitivity on a 0-10 scale, with a higher score associated with greater pain sensitivity. The PSQ, which has been validated in normal and chronic pain populations, has never been used in ocular surface research [89,99,104–107].

PSQ: Pain Sensitivity Questionnaire

This questionnaire contains a series of questions in which you should imagine yourself in certain situations. You should then decide if these situations would be painful for you and if yes, how painful they would be. **Let 0 stand for no pain; 1 is an only just noticeable pain and 10 the most severe pain that you can imagine or consider possible.** Please mark the scale with a circle on the number that is most true for you. Keep in mind that there are no "right" or ''wrong" answers; only your personal assessment of the situation counts.

Please try as much as possible not to allow your fear or aversion of the imagined situations affect your assessment of painfulness.

1. Imagine you bump your shin badly on a hard edge, for example, on the edge of a glass coffee table.
   How painful would that be for you?
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

2. Imagine you burn your tongue on a very hot drink.
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

3. Imagine your muscles are slightly sore as a result of physical activity.
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

4. Imagine you trap your finger in a drawer.
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

5. Imagine you take a shower with lukewarm water.
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

6. Imagine you have mild sunburn on your shoulders.
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

7. Imagine you grazed your knee falling off your bicycle.
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

8. Imagine you accidently bite your tongue or cheek badly while eating.
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

9. Imagine walking across a cool tiled floor with bare feet.
   0= not at all painful 10= most severe pain imaginable
   0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

10. Imagine you have a minor cut on your finger and inadvertently get lemon juice in the wound.
    0= not at all painful 10= most severe pain imaginable
    0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

11. Imagine you prick your fingertip on the throne of a rose.
    0= not at all painful 10= most severe pain imaginable
    0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

12. Imagine you stick your bare hands in the snow for a couple of minutes or bring your hand in contact with snow for some time, for example, while making snowballs.
    0= not at all painful 10= most severe pain imaginable
    0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

13. Imagine you shake hands with someone who has a normal grip.
    0= not at all painful 10= most severe pain imaginable
    0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

14. Imagine you shake hands with someone who has a very strong grip.
    0= not at all painful 10= most severe pain imaginable
    0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

15. Imagine you pick up a hot pot by inadvertently grabbing its equally hot handles.
    0= not at all painful 10= most severe pain imaginable
    0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

16. Imagine you are wearing sandals and someone with heavy boots steps on your foot.
    0= not at all painful 10= most severe pain imaginable
    0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

17. Imagine you bump your elbow on the edge of a table ("funny bone").
    0= not at all painful 10= most severe pain imaginable
    0 ----------1 ---------- 2 ---------- 3 ---------- 4 ---------- 5 ---------- 6 ---------- 7 ---------- 8 ----------9 ---------- 10

Figure 5-1 The Pain Sensitivity Questionnaire

The purpose of this study was to determine if the PSQ score is associated with common subjective instruments for assessing ocular discomfort and dryness symptoms related to dry eye and CL discomfort. We hypothesize that a higher PSQ score (i.e., greater sensitivity to pain) is associated with greater ocular discomfort reported, even after adjustment for any other significant factors. This study could further our understanding of how pain sensitivity may be a factor contributing to the the discrepancy between signs and symptoms of ocular discomfort: a patient with greater pain sensitivity may report symptoms in the absence of any clinical signs, while a less sensitive patient may suffer little or no discomfort in spite of visible ocular surface pathology. Furthermore, an awareness of the role of pain sensitivity in patient symptomatology could inform clinician diagnostic and treatment decisions in personalized eye care.

5.3 METHODS

5.3.1 Subjects

Subjects were recruited from the University of California, Berkeley and the surrounding community. Subjects taking systemic or ocular medication, or with a history of systemic or ocular disease or surgery, were excluded from the study. Subjects were also excluded if they were smokers, or currently or previously pregnant. Contact lens wearers (CLW) and non-contact lens wearers (non-CLW) were recruited for the study; non-CLWs were defined as individuals that had never worn CLs before or had discontinued CLs more than one year prior to the study.

The study population consisted of individuals who were of either Asian or Caucasian descent. These two groups of subjects were chosen because previous research has demonstrated inter-ethnic differences in pain sensitivity [91,160], and in both subjective and objective responses to CLs [155,158,161–165]. Individuals were considered to be of Asian ethnicity if they were of Chinese, Korean, Vietnamese or Taiwanese descent, and of Caucasian ethnicity if they were of European descent. Individuals of mixed ethnicity were excluded from the study. Subjects were instructed to refrain from using any eye makeup or eye drops on the day of the visit. Informed consent, with a complete description of the goals, risks, benefits and procedures of the study, was obtained from all participants. This study observed the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley Committee for Protection of Human Subjects.

5.3.2 Instrumentation and Procedures

Subjects were administered a baseline questionnaire battery composed of the OSDI, the PSQ, the Dry Eye Flow Chart (DEFC), a set of 100-point visual analog rating scales (VAS) for average and end-of-day (EOD) comfort (0=poor comfort, intolerable, 100=excellent comfort, cannot be felt), frequency of discomfort on average and at EOD (0=Never, 100=All the time), average and EOD dryness (0=no sensation of dryness whatsoever, 100=extremely dry, intolerable), and frequency of dryness on average and at EOD (0=Never, 100=All the time). In addition, a demographics and history

questionnaire was administered that included items for age, gender, ethnic group (Asian, Caucasian), immigration status (born in the United States or immigrated) and current or past CL wear [158]. The questionnaire battery took approximately 20 minutes to complete and the order of the questionnaires was randomized to minimize any potential bias due to the effects of test fatigue.

In addition to determining whether the PSQ score is significantly related to the aforementioned measures of ocular discomfort, a second goal of the study was to determine whether differences in subjects' pain sensitivities, as measured by the PSQ, can be shown to partly explain the relatively small differences in comfort and dryness between fellow eyes due to differences in lens fit; therefore, an issue faced during the design of this study was the development of a method to induce such an inter-eye difference. We opted to fit all subjects with a single brand of CL in a single base curve and power (Air Optix Night and Day [B.C. 8.6, Power -1.50 DS]) for both eyes, with one inverted and one normally oriented CL inserted contralaterally based on random assignment. Thus, a relatively small range of differences in discomfort and dryness due solely to differences in lens fit would be induced, eliminating the possibility of more drastic differences we felt may occur in some subjects with different lens designs, surface coatings or soaking solutions. Subjects who indicated a strong baseline preference (on a 5-point Likert scale) for one eye or the other prior to study CL wear were excluded, so that any inter-eye differences in comfort and dryness would be due solely to the different CL fits.

An anterior segment examination under white light was performed prior to CL insertion to ensure there was no evidence of active or pre-existing ocular pathology (e.g., corneal scars, infiltrates, excessive corneal epithelial irritation). Subjects wore the normally oriented and inverted CLs contralaterally for 30 minutes, during which time they completed VAS ratings of comfort and dryness every 5 minutes. Subjects were masked as to which eye received the inverted CL, and were instructed that they could have the CLs removed at any time as they wished. After 30 minutes, a slit lamp examination with fluorescein was performed to assess CL wettability, post-blink movement, tightness and centration. The methods for CL assessment are described further in Tan et al. [155]

5.3.3 Statistical Methods

The PSQ provides three numerical values: the overall pain sensitivity score (PSQ-Total), and scores for sensitivity to situations with minor (PSQ-min) and moderate (PSQ-mod) pain. The PSQ scores were highly correlated, in agreement with previous studies, and we found through preliminary exploratory analysis (not shown) that the minor pain (PSQ-min) score best reflected the discomfort experienced with dry eye and CL wear [92,99]. Therefore, this analysis will focus on the PSQ-min score; we will refer to the PSQ-min score as the "PSQ score" for the remainder of the manuscript.

After a thorough exploratory and descriptive analysis, baseline questionnaire responses to the OSDI, DEFC, and VAS for average and EOD comfort and dryness (severity and frequency) were modeled as functions of the PSQ score, adjusted for any other significant subject characteristics including age, gender, ethnicity (Asian, Caucasian), immigration status (United States-born, immigrated), history of CL wear,

time awake prior to the examination, palpebral aperture size, and presence of grade 2 or greater corneal staining in either eye with white light. Our goal in building such models was to determine whether, after adjusting for any factors that may be related to comfort or dryness outcomes, the PSQ score would remain an additional significant explanatory factor.

After modeling the baseline subjective outcomes, we examined the paired-eye data from 30 minutes of contralateral wear of one normally-oriented and one inverted soft CL, during which time VAS ratings of comfort and dryness for each eye were made by the subject every 5 minutes. We modeled the inter-eye differences (inverted – normally-oriented) in ratings of comfort (IED-C) and dryness (IED-D) as linear mixed effects models, in order to account for the potential within-subject correlations between fellow eyes and over repeated measurements. The candidate explanatory (fixed effects) variables we examined included PSQ score, baseline subject characteristics and baseline symptom ratings, as well as post-wear CL wettability, movement, push-up test tightness, and decentration.

For both the baseline and post-CL wear analyses, the best models were selected based on consideration of F-test p-values, examination of residual and other diagnostic plots, and comparison of the log-Likelihood for nested models or Akaike's Information Criterion for non-nested models. A subset of subjects felt that the inverted CL was at times more comfortable than the normally oriented CL; because we were not testing hypotheses about inverted-vs.-normally-oriented CLs, but rather simply inverting one lens to create some difference in subjective sensation, we elected to model the absolute values of the IED-C and IED-D. In addition, many subjects found it difficult to provide ratings of comfort or dryness during the initial period of CL settling, which was reflected in excessively high within- and between-subject variability in the first 10 minutes; we therefore elected to analyze subject ratings made between 10 and 30 minutes post-insertion, after the lens had settled. Finally, in order to better approximate normality, we modeled both IED-C and IED-D on the natural log scale.

5.4 RESULTS

5.4.1 Subject Characteristics

A sample of 168 subjects was initially recruited for the study. Fourteen subjects did not complete the study due to pre-existing corneal scar, mixed ethnicity, or a strong baseline comfort or dryness preference for one eye over the other. One subject was unable to complete all study measurements due to inability to tolerate 30 minutes wear of the study CLs. Further details on the reasons for disqualification and dropout are provided in Table 5-1.[166] A total of 153 subjects (40 male, 113 female) with a mean (SD) age of 22.6 (3.4) years (range: 18-34 years) successfully completed the study. The study cohort was composed of 91 Asians and 62 Caucasians; 114 subjects were born in the United States and 39 subjects immigrated to the United States (85% of subjects who immigrated were Asian). Ninety subjects were experienced CLW and 63 were non-CLW.

Subjects Recruited	**168**
Failed to Meet Eligibility Criteria	**8**
Mixed Ethnicity	4
Pre-existing Corneal Scar	2
History of Iritis	1
Unable to Document Recent Eye Exam	1
Disqualified	**6**
Unable to Insert CLs	3
Strong Baseline Comfort Preference	3
Dropouts	**1**
Unable to Complete 30min CL Wear	1
Total Failing to Enter and Complete Study	**15**
Total Successfully Completing Study	**153**

Table 5-1 Disposition of all recruited subjects at end-of-study.

5.4.2 PSQ Score

The mean (SD) PSQ score was 2.7 (1.3) with a range of 0.1 to 7.1. Figure 5-2 depicts the PSQ scores stratified on gender, ethnicity, immigration status and CL history. As shown on Figure 5-2, there was no significant difference ($p=0.229$) in mean PSQ score between men (2.5) and women (2.8), nor was there a significant difference ($p=0.331$) between CLW (2.8) and non-CLW (2.6). Asians had a higher mean PSQ score (3.0) than did Caucasians (2.3), indicating significantly greater pain sensitivity on average among Asians ($p<0.001$). Subjects who immigrated to America also had a higher mean PSQ score (3.1) than those born in America (2.6; $p=0.021$).

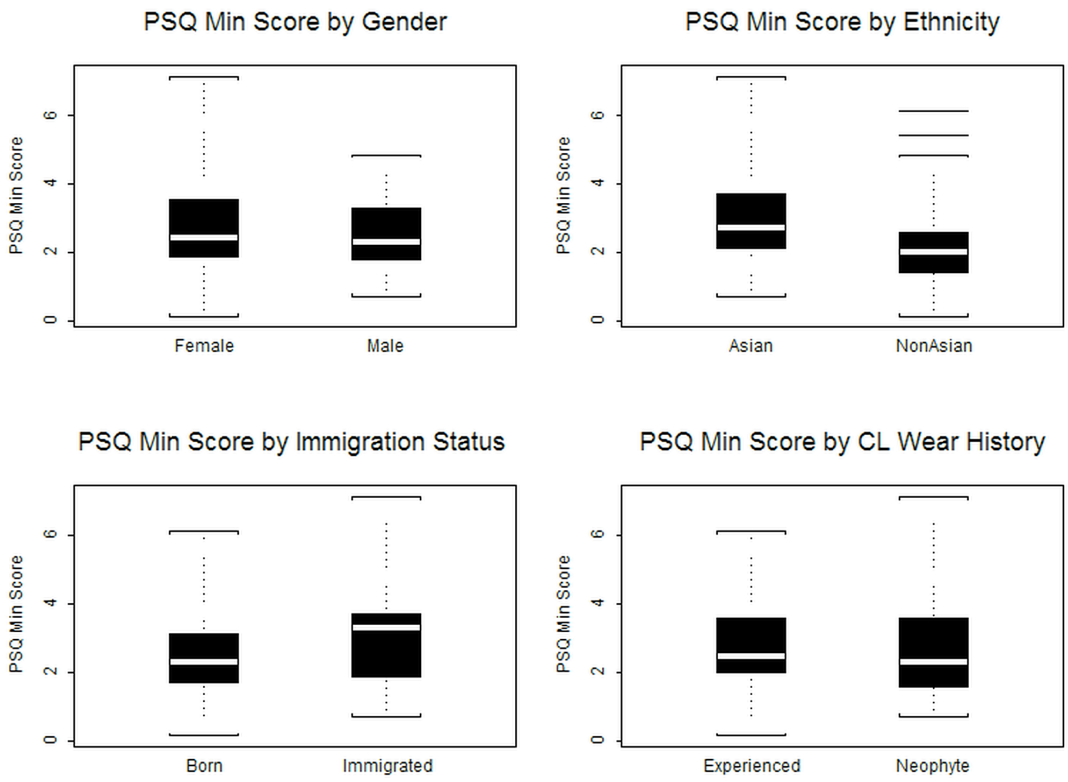

Figure 5-2 PSQ score stratified on gender, ethnicity, immigration status, and CL wearing history.

5.4.3 Baseline Questionnaire Response

Descriptive statistics for the baseline questionnaire responses are shown in Table 5-2. In multivariable linear mixed effects models (Table 5-3), a higher OSDI score was significantly associated with higher PSQ score (p=0.005), as well as with female gender (p=0.016) and Caucasian ethnicity (p=0.004). There was an estimated 11 point greater OSDI score for the highest (7.1) vs. the lowest (0.1) PSQ scores observed. Lower average comfort was significantly associated with higher PSQ score (p=0.005), as well as with CLW (p=0.009). There was an estimated 20 unit lower average comfort for the highest vs. the lowest PSQ scores observed. A greater frequency of discomfort on average was significantly associated with CLW (p=0.015) and a higher PSQ score (p=0.009), with an estimated 17 unit higher rating for the highest vs. the lowest PSQ scores observed. Lower EOD comfort was also significantly associated with CLW (p<0.001) and a higher PSQ score (p=0.001), with an estimated 31 unit lower EOD comfort rating for the highest vs. the lowest PSQ scores observed. A greater frequency of EOD discomfort was significantly associated with being a female (p=0.009) and CLW (p<0.001), but not significantly associated with PSQ score (p=0.379).

	Min	Max	Median	Mean	SD
PSQ Score	0.14	7.14	2.43	2.69	1.25
OSDI	0.00	45.83	6.25	8.63	8.60
DEFC	1	5	2	2.4	1.4
Avg Comfort	27	99	87.0	81.3	16.9
Avg Discomfort Freq	0	75	9.0	13.7	15.8
EOD Comfort	6	99	75.0	69.8	25.0
EOD Discomfort Freq	0	99	15.0	25.7	27.3
Avg Dryness	0	72	12.0	18.7	19.7
Avg Dryness Freq	0	75	9.0	16.3	18.3
EOD Dryness	0	87	18.0	27.2	26.6
EOD Dryness Freq	0	90	15.0	25.8	27.5

Table 5-2 Descriptive statistics for baseline questionnaire responses.

Outcome	Intercept	PSQ	Gender: Male	Ethnicity: Caucasian	CLWHx: neophytes
OSDI	3.18	1.59 p= 0.002	-3.25 p= 0.016	3.34 p= 0.004	
Avg Comfort	85.93	-2.81 p= 0.005			7.02 p= 0.009
Avg Discomfort Frequency	9.78	2.41 p= 0.009			-6.15 p= 0.015
EOD Comfort	74.10	-4.33 p= 0.001			17.94 p= <0.001
EOD Discomfort Frequency	32.87	0.65 p= 0.379	-11.08 p= 0.009		-14.54 p=<0.001
Avg Dryness	25.27	0.41 p= 0.181		-7.91 p= 0.020	-10.83 p<0.001
Avg Dryness Frequency	23.34	0.07 p= 0.251		-8.75 p= 0.006	-8.97 p= 0.002
EOD Dryness	31.38	2.32 p= 0.040	-8.01 p= 0.020		-20.33 p= <0.001
EOD Dryness Frequency	-10.43	1.89 p= 0.242	-10.13 p= 0.006		-21.11 p =<0.001
DEFC Score	3.19	0.03 p=0.069		-0.45 p=0.047	1.62 p<0.001

Table 5-3 Separate multivariate models showing the associations between subject characteristics and subjective responses to the baseline questionnaires. The arbitrary reference groups for Gender, Ethnicity and CLWHx (CL Wear History) were Female, Asian and Experienced, respectively. A higher value in average or EOD comfort is associated with greater comfort. A higher value in average or EOD dryness is associated with greater dryness.

Higher average dryness severity was significantly associated with being Asian (p=0.020) and CLW (p<0.001), but not with PSQ score (p=0.181). A greater frequency of dryness on average was also significantly associated with being Asian (p=0.006) and CLW (p=0.002), but not with PSQ score (p=0.251). Higher EOD dryness severity was significantly associated with higher PSQ score (p=0.041), as well as with female gender (p=0.020) and CLW (p<0.001). There was an estimated 16 unit higher EOD dryness severity rating for the highest vs. the lowest PSQ scores observed. A greater frequency of EOD dryness was significantly associated with greater age (p=0.001), being female (p=0.006) and CLW (p<0.001), but not with PSQ score (p=0.242). A higher DEFC score was significantly associated with being Asian (p=0.047) and CLW (p<0.001), and although the PSQ score approached significance at the α=0.05 level (p=0.069) the

effect size was clinically insignificant, with an estimated difference of 0.2 units on the 5-unit DEFC scale between the highest and lowest PSQ scores observed.

5.4.4 Subjective Response During 30 min Contact Lens Wear

Descriptive statistics for the CL fitting characteristics are shown in Table 5-4. There was no significant difference in wettability between the inverted and normally oriented CLs (p=0.893). The inverted CL demonstrated more movement after a blink than the normally oriented CL (p<0.001). The inverted CL, on average, showed less lens tightness than the normally oriented CL (p<0.001).

	Inverted CL	Normally-Oriented CL	p-value
Wettability	3.59 (0.43)	3.59 (0.44)	0.893
Movement (in mm)	0.62 (0.47)	0.31 (0.26)	<0.001
Tightness	41.1 (8.5)	52.2 (9.4)	<0.001

Table 5-4 Mean (SD) and paired t-test p-values for fitting characteristics of the normally-oriented and inverted CLs.

There was a decrease in IED-C when comparing the values at 10 and 30 minutes post-insertion (14.0 vs. 11.6, respectively; p=0.02) but with an estimated difference in VAS rating of less than 3 units on the 100-point scale, it was not clinically significant. There was no significant difference in IED-D when comparing the values at 10 and 30 minutes post-insertion (6.5 vs. 6.3, respectively; p=0.73). Therefore, in comparing VAS ratings to PSQ scores, the means of the IED-C and IED-D over the twenty-minute measurement period were used for the reminder of the analysis.

Age, gender, ethnicity, immigration status, CLW history, time awake, CL wettability and movement were all found not to be significantly related to the IED-C. The linear mixed effects model showed that a greater comfort difference between fellow eyes was significantly associated with a higher PSQ score (p=0.013). There was an estimated 7 unit increase in the IED-C rating for the highest vs. the lowest PSQ scores observed.

Gender, ethnicity, CLW history, time awake, CL wettability, movement, and tightness were all found not to be significantly related to the IED-D. The linear mixed effects model showed that a greater dryness difference between fellow eyes was significantly associated with a higher PSQ score (p=0.010). There was an estimated 7 unit increase in the IED-D rating for the highest vs. the lowest PSQ scores observed.

5.5 Discussion

In this study we found that the PSQ provides a clinically relevant insight into the perception of symptoms of ocular dryness and discomfort. Examining the statistical models, the PSQ score appears to have a significant independent effect on subjective

ratings of ocular comfort and dryness, even after adjusting for significant subject demographic and ocular characteristics. As pain sensitivity is based on how painful stimuli are rated, it is not surprising that the PSQ was primarily associated with the severity and not the frequency of ocular discomfort and dryness. We believe that with further work, the PSQ could be employed to provide a deeper insight into ocular discomfort and dryness. As a key example, one of the most confounding aspects of dry eye is the sporadic and unreliable correlation between signs and symptoms of dry eye [29,77]. It is not uncommon for individuals to have the same clinical presentation of dry eye but have vastly different OSDI scores; conversely, very similar OSDI scores can be observed with vastly different clinical signs. Such discrepancies may be explained in part by pain sensitivity either amplifying (in an individual with high sensitivity to pain) or weakening (in an individual with low sensitivity to pain) the perception of dry eye symptoms. A patient with greater pain sensitivity may suffer symptoms of discomfort when ocular surface pathology that could lead to such symptoms is sub-clinical. A less sensitive patient may report no symptoms at all, even when the clinician can clearly identify signs of desiccation and damage to the ocular surface.

At the current time these results are suggestive only, as the purpose of this study was not to directly examine the relationship between DE signs and symptoms, but to determine whether the PSQ could be used to quantify the effect of individual pain sensitivity on ratings of subjective symptoms. Further suggestion of the potential for utilizing the PSQ in future studies can be seen with CL discomfort in this study, for which the PSQ score was the only significant explanatory variable (no CL fitting characteristics were found to be significant). With the PSQ score now established as an independent, significant explanatory factor in our models of several different subjective assessments of ocular discomfort and dryness, future work will include examining the relationship between signs and symptoms in subjects with a wide range of pain sensitivities.

Although Vehof et al. reached a similar conclusion to this study, the logistical challenges of experimentally measuring pain sensitivity as was done in that study limit its clinical and research utility [159]. Experimentally measuring pain poses many issues and it requires the development of a complex plan to measure pain sensitivity, accounting for the type of pain modality used (heat, cold or pressure) and specifications for testing (i.e., strength, placement and timing of stimuli delivery) [92,99]. In addition, inducing pain or the fear of pain-inducement can have a significant cognitive effect on subjects, potentially confounding how they perceive an irritant or answer subjective questionnaires [151–153]. The PSQ overcomes many of these challenges and the noted advantages of the PSQ over experimental pain sensitivity assessments could make it a useful tool in our efforts to better understand ocular symptomatology.

An acknowledgement of the role that pain sensitivity has in influencing ocular discomfort is important because of the limited development in treatment options for dry eye (Restasis® is the only FDA-approved medication for dry eye), which is partially due to the lack of association between signs and symptoms of dry eye [45]. Fifteen companies have sought and failed to get FDA approval for their dry eye drugs; several drugs, most recently Eleven Biotherapeutics' EBI-005, were unable to pass Phase 3 clinical trials due to inability to show an improvement in signs *and* symptoms. Meeting both prerequisites was not required when Restasis® was FDA approved, but now fulfilling

these two criteria is hampered by low repeatability and poor correlation with symptoms seen in current diagnostic tests [18,45,47,48]. Even if test repeatability was improved, there may still be a discrepancy between signs and symptoms due to the impact that pain sensitivity has on their relationship, as suggested by this study. Further, as the minimal clinically important difference for the OSDI ranges from 7.0 to 9.9, which is within the effect size seen in this study (11 points, when comparing the subjects with the least and most sensitivity to pain), this suggests that pain sensitivity should be used as a corrective factor when assessing improvements of signs and symptoms in future clinical trials [167].

This study found that the PSQ was associated with factors such as ethnicity (Asians having greater pain sensitivity) and immigration status (immigrants having greater pain sensitivity), which is in agreement with previous studies [91,168,169]. Gender was not found to be associated with PSQ score, which is consistent with other studies [92,99]. In this study, subjects of European-Caucasian descent were associated with a greater OSDI score, which is surprising as subjects of Asian descent were associated with greater EOD dryness and because Asians have been reported to have a greater prevalence of dry eye [3,4]. The discrepancy may be due to sampling variation but it may also be important to consider the inherent difference between the two questionnaires. The EOD dryness VAS consists of one question, “How would you rate the dryness of each eye at the end of the day?” This is in contrast to the OSDI, which has twelve questions that show significantly greater linguistic complexity compared to the VAS. It is possible that Asians, with a third being immigrants, may respond differently (i.e., report less dryness) in a complicated questionnaire compared to a simpler one. This finding highlights the need for further improvements in our understanding of inter-ethnic differences in dry eye.

Comparing subjects with the least and greatest sensitivity to pain (as measured by the PSQ), the inter-eye differences in comfort and dryness were estimated to be approximately 7 points on the 100-point VAS, which is a relatively small but clinically significant difference. Nevertheless, it is possible that this study may offer clues as to why some patients, after years of being asymptomatic CL wearers, suddenly become symptomatic, even without evident clinical signs. The risk of developing dry eyes and CL intolerance increase with age, likely due to alterations to the tear film and ocular surface that occur over time [170,171]. It is possible that minor alterations to the tear film/ocular surface, which may not be considered clinically significant, that occur with age cause symptoms to be magnified in individuals with greater pain sensitivity, leading to CL dropout. This is supported by studies that have found no difference in tear film properties between symptomatic and asymptomatic CLW; the exception being conflicting reports on patients with lid wiper epitheliopathy and patients with conjunctival folds [156,172,173]. The results from this study suggest that a cross-sectional study — and eventually a longitudinal study — is warranted to determine if increased pain sensitivity is a risk factor for the discontinuation of CL wear.

5.6 Conclusion

Using the PSQ, we were able to show that pain sensitivity was related to perception of ocular comfort and dryness. Additionally, pain sensitivity was found to be associated with the subjective assessment of inter-eye differences in comfort and dryness during CL wear. The results suggest that pain sensitivity must be considered when interpreting subjective responses to symptom-related questionnaires. Pain sensitivity differences may also offer a partial explanation for the discrepancy seen between the signs and symptoms of ocular discomfort, including dry eye and CL intolerance or dissatisfaction.

Chapter 6: Conclusion

A major impediment in developing novel treatments for EDE has been the inability to understand the mechanism that causes ocular discomfort when tear-film instability occurs. IRT offers a way to assess tear-film instability in an objective and non-invasive manner. In addition, the test directly measures OSC and provides indirect information on how areas of hyperosmolarity develop (as OSC and FTBU are co-localized).[122] Chapters 1 and 2 of the thesis suggest that IRT demonstrated characteristics that potentially made it a possible gold standard test for EDE as it was repeatable, objective, and provides an easily interpretable metric, the OSC rate, which could be used to monitor and educate patients with EDE. The IRT may overcome one of the major issues with current EDE tests, which is the poor association between signs and symptoms.[29,46,70] The use of IRT could provide a metric for tear-film instability (defined by temperature) and provide an insight into how it contributes to TRPM-8 activation, as suggested by Chapter 3 of the thesis.

Nevertheless, even if IRT measures ocular surface irritation, it may not translate to understanding how it is ultimately perceived, as pain sensitivity is highly individualized.[80–82] It would not be unexpected if an identical ocular irritant, applied to a group of individuals, led to a diverse range in the level of discomfort reported.[80,83,84] Therefore, a link between tear-film instability and EDE symptoms may not be found unless pain sensitivity is taken into account. Only limited work had been done on pain sensitivity and ocular discomfort, as experimentally measuring pain sensitivity is difficult and inducing pain on subjects can have a profound impact on how they perceive an irritant or answer questionnaires.[151–153] The PSQ overcomes these challenges and could be an effective tool in clinical studies on EDE as suggested by Chapter 4 of the thesis.

Future work will be directed by the questions that were posed by the results of the thesis. The first issue is on what is the most appropriate method on interpreting the OST and OSC rate measurements. Although current literature suggests a strong correlation between OST and TERs,[55,56,64,66,67,122,174] no simple methodology currently exists to quantify TERs.[6,23,49,50,175–177] Several authors have attempted to diagnose human dry-eye disease by measuring OST versus time, fitting a line to the data, and reporting an average cooling rate presumed to be correlated with TER.[58,64,128,131,178] However, the cooling profile is not a linear but rather an exponential decay, and the use of linear approximation inaccurately portrays the cooling. More importantly, these cooling rates fail to account for core body temperatures and environmental conditions such as ambient temperature and humidity.[67,114,124] Thus, the reported cooling rates are a strong function of environmental heat losses independent of TERs.

Recently, Tan et al.[54] developed a heat-transfer model to isolate the evaporative contribution to ocular cooling and extract an overall evaporation rate by averaging over a single blink using an average region of interest on the ocular surface. However, their overall evaporation rate does not account for variations in local evaporation rates that occur when regions of the TFLL undergo breakup and expose the underlying aqueous to the environment, resulting in decreased local mass transfer resistances and increased local evaporation rates through lipid-free areas.[23,25] As the TFLL is thought to

inhibit evaporation by approximately 75-90%, a region of breakup can play a significant role in the TER, but this has never been previously investigated due to the inability to directly identify or assess these areas.[21,24,179]

Recently, Li et al.[122] employed a simultaneous assessment of OST and fluorescein tear breakup (FTBU), which demonstrated that areas of FTBU and regions of cooling were co-localized, and that cooling was noted one to two seconds before an area of FTBU was observed. This implies that OST measurement indirectly assessed the active process of tear film evaporation while FTBU was an end point resulting from a tear film that is thinned enough by evaporation to cause fluorescence quenching.[32,62,117,118,121] The results are consistent with a mathematical model developed by Peng et al., which theorized an intact TFLL would decrease TER.[25] Therefore, a requirement for FTBU formation is an area of TFLL breakup that increases TER, which is observed as an area of cooling. The locally elevated evaporation rate causes a deepening fissure in the tear film, which when sufficiently thin causes FTBU.[25]

The next issue is on what areas of the cornea should we focus on when we assess OST and OSC rate. All the research, including our own work, calculates the OSC rate by assessing the OST averaged over the entire cornea because it is the simplest method.[58,180,181] This was considered to be adequate until new research suggested that the ability to discern what occurs in localized regions of elevated tear evaporation is more important than knowing the general evaporation rate.[25,76] In addition, if the area of increased OSC is small, then considering the OSC rate over the entire cornea will underestimate it. An example can be seen in Figure 6-1 and 6-2, where the general OSC rate (0.13^0C/sec) is half the localized OSC rate (0.26^0C/sec). As a result, significant information would be lost if we fail to account for localized regions of OSC. Understanding these areas of OSC are important as they represent regions of tear-film instability. Therefore, adjustments in the hardware and software may need to be implemented to allow for more accurate temperature assessment in localized regions of the ocular surface.

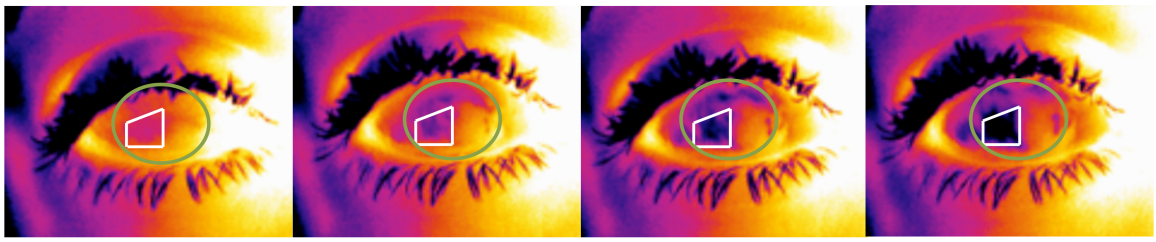

Figure 6-1 Region of interest designated to provide general OSC (green circle) and localized OSC (white quadrilateral; where most cooling is occurring)

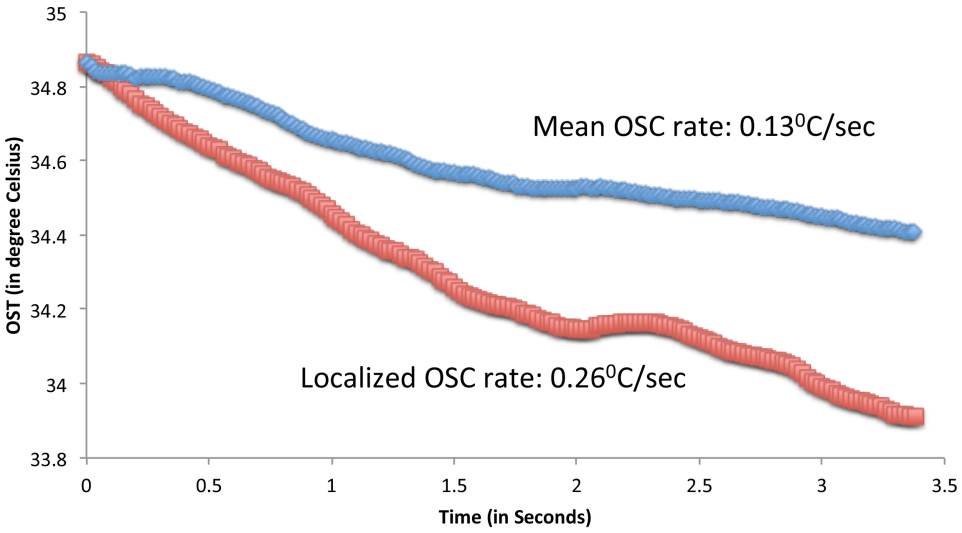

Figure 6-2 On the plot general OSC (in blue) is based on the area marked by the green circle in Figure 6-1. Localized OSC (in red) is based on the area marked by the white quadrilateral in Figure 6-1.

As stated earlier in the thesis, one compelling aspect of using IRT to assess tear-film instability is the potential ability to objectively measure the magnitude of TRPM-8 stimulation on the ocular surface. Nevertheless, there are significant questions regarding what aspects of tear-film instability are key to activating the receptors. To discern what features may be important, a consideration should be made on how nociceptors are activated and the afferent pathway used for transmitting sensory information. Nociceptors are activated by high-level stimuli, which makes the receptors well designed to sense noxious stimuli.[30,182] Once the nociceptors are activated, the signal is transmitted along the afferent pathway to the dorsal horn for nociceptive processing. At this stage, the signal, combined with the signal from neighboring nociceptors, undergoes spatial and temporal summation, contributing to pro- or antinociception.[30,182] This information is then relayed to regions in the brain.

Most of what we understand regarding ocular discomfort is based on animal *in vivo* models but the use of a human *in vivo* model is essential because it would allow us to answer questions that animal models cannot. Animal *in vivo* models measure TRPM-8 activation using extracellular single-unit recording of single neurons in the trigeminal ganglion, which limits assessment to a handful of receptors.[183,184] Most importantly, following the pathway for nociceptor signaling, the location of measurement (trigeminal ganglion) is before the dorsal horn of the spine, where nociceptive processing occurs.[30,185] The dorsal horn is an important site because it is where the regulation of extracellular signal-regulated protein kinase (e.g., mitogen-activated protein kinase, protein kinase C) either upregulate (pronociceptive) or downregulate (antinociceptive) the signal.[30,185] Therefore, only measuring the trigeminal ganglion will prevent an understanding to the important role that nociceptive processing has on influencing ocular discomfort.

For the future work, it will be inferred nociceptor based on the discomfort that subjects report, which has been used in other studies.[30,186] This assumption has two major issues: the discomfort experienced could represent activation of other polymodal nociceptors and perception of discomfort varies between individuals. Although cooling and hyperosmolarity will activate other polymodal nociceptors, the TRPM-8 are still the predominant group of receptors that respond to it.[72] The issue of inter-subject variation in pain perception will be considered using the PSQ.

Based on this understanding of the nociceptors, the IRT can be used to answer the following questions on what features of tear-film instability (assessed in terms of OSC) are important for activating TRPM-8 receptors: (1) what is the threshold for OSC needed to activate the TRPM-8 receptors, (2) does the area size of OSC influence ocular discomfort (spatial summation), (3) does the time exposed to OSC influence ocular discomfort (temporal summation), and (4) what role does conjunctival cooling, which has TRPM-8 receptors, play on discomfort. We will use the hardware and software changes in helping to characterize these features of OSC more accurately.

An issue posed by Chapter 4 of the thesis is related to one of the most confounding aspects of dry eye is the sporadic and unreliable correlation between signs and symptoms of dry eye [29,77]. It is not uncommon for individuals to have the same clinical presentation of dry eye but have vastly different OSDI scores; conversely, very similar OSDI scores can be observed with vastly different clinical signs. Such discrepancies may be explained in part by pain sensitivity either amplifying (in an individual with high sensitivity to pain) or weakening (in an individual with low sensitivity to pain) the perception of dry eye symptoms. A patient with greater pain sensitivity may suffer symptoms of discomfort when ocular surface pathology that could lead to such symptoms is sub-clinical. A less sensitive patient may report no symptoms at all, even when the clinician can clearly identify signs of desiccation and damage to the ocular surface. With the PSQ score now established as an independent, significant explanatory factor in our models of several different subjective assessments of ocular discomfort and dryness, future work will include examining the relationship between signs and symptoms in subjects with a wide range of pain sensitivities.

If the hypothesis is accurate, subjects with greater pain sensitivity will report more discomfort and dryness (subjective response) and have a shorter MIBP (objective response) when controlling for OSC rate in the linear mixed effects model. In addition, the analysis could determine how PSQ score should be mathematically accounted for when examining the relationship between OSC rate and the discomfort experienced (i.e., in the most basic form, OSC rate + PSQ score = perception of discomfort), potentially useful in future clinical trials.

Further understanding of how tear-film instability induces ocular discomfort is needed. IRT may provide a potential tool for discerning this relationship but to accomplish this, we need to optimize the IRT to better assess localized regions of OSC, determine what characteristics of tear-film instability contribute to ocular discomfort, and ascertain how pain sensitivity affects the perception of tear-film instability. This future work will require a unique approach in measuring the magnitude of ocular surface irritation and consider how pain sensitivity influences the perception of discomfort when tear film instability occurs. The achievement of this future work using innovative

approaches could reveal the underlying mechanisms for which tear film instability contributes to ocular discomfort and help the millions of people with EDE.

Bibliography

1. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. Arch Ophthalmol 2000;118(9):1264-1268.
2. Lee a J, Lee J, Saw S-M, et al. Prevalence and risk factors associated with dry eye symptoms: a population based study in Indonesia. Br J Ophthalmol 2002;86(12):1347-1351.
3. Galor A, Feuer W, Lee DJ, et al. Prevalence and risk factors of dry eye syndrome in a United States veterans affairs population. Am J Ophthalmol 2011;152(3):377-384.e2.
4. Jie Y, Xu L, Wu YY, Jonas JB. Prevalence of dry eye among adult Chinese in the Beijing Eye Study. Eye (Lond) 2009;23(3):688-693.
5. Gayton JL. Etiology, prevalence, and treatment of dry eye disease. Clin Ophthalmol 2009;3:405-412.
6. Goto E, Endo K, Suzuki A, Fujikura Y, Matsumoto Y, Tsubota K. Tear evaporation dynamics in normal subjects and subjects with obstructive meibomian gland dysfunction. Investig Ophthalmol Vis Sci 2003;44(2):533-539.
7. Ozcura F, Aydin S, Helvaci MR. Ocular surface disease index for the diagnosis of dry eye syndrome. Ocul Immunol Inflamm 2007;15(5):389-393.
8. Blackie C a, Korb DR, Knop E, Bedi R, Knop N, Holland EJ. Nonobvious obstructive meibomian gland dysfunction. Cornea 2010;29(12):1333-1345.
9. Sakane Y, Ohashi Y, Ohashi Y. Development and Validation of the Dry Eye–Related Quality-of-Life Score Questionnaire. JAMA … 2013:E1-E8.
10. Mizuno Y, Yamada M, Miyake Y. Association between clinical diagnostic tests and health-related quality of life surveys in patients with dry eye syndrome. Jpn J Ophthalmol 2010;54(4):259-265.
11. Meijer JM, Meiners PM, Huddleston Slater JJR, et al. Health-related quality of life, employment and disability in patients with Sjogren's syndrome. Rheumatology (Oxford) 2009;48(9):1077-1082.
12. Li M, Gong L, Sun X, Chapin WJ. Anxiety and depression in patients with dry eye syndrome. Curr Eye Res 2011;36(1):1-7.
13. Labbé A, Wang YX, Jie Y, Baudouin C, Jonas JB, Xu L. Dry eye disease, dry eye symptoms and depression: the Beijing Eye Study. Br J Ophthalmol 2013;97(11):1399-1403.
14. Kim KW, Han SB, Han ER, et al. Association between depression and dry eye disease in an elderly population. Invest Ophthalmol Vis Sci 2011;52(11):7954-7958.
15. Rosenfield M. Computer vision syndrome: a review of ocular causes and potential treatments. Ophthalmic Physiol Opt 2011;31(5):502-515.
16. Yamada M, Mizuno Y, Shigeyasu C. Impact of dry eye on work productivity. Clinicoecon Outcomes Res 2012;4:307-312.
17. Yu J, Asche C V, Fairchild CJ. The economic burden of dry eye disease in the United States: a decision tree analysis. Cornea 2011;30(4):379-387.
18. Schaumberg D a, Nichols JJ, Papas EB, Tong L, Uchino M, Nichols KK. The international workshop on meibomian gland dysfunction: report of the subcommittee on the epidemiology of, and associated risk factors for, MGD.

Invest Ophthalmol Vis Sci 2011;52(4):1994-2005.
19. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 2007;5(2):75-92.
20. Knop E, Knop N, Millar T, Obata H, Sullivan D a. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. Invest Ophthalmol Vis Sci 2011;52(4):1938-1978.
21. Iwata S, Lemp M a, Holly FJ, Dohlman C. Evaporation rate of water from the precorneal tear film and cornea in the rabbit. IOVS 1969;8(6):613-619.
22. Mathers W. Evaporation from the ocular surface. Exp Eye Res 2004;78(3):389-394.
23. Cerretani CF, Ho NH, Radke CJ. Water-evaporation reduction by duplex films: application to the human tear film. Adv Colloid Interface Sci 2013;197-198:33-57.
24. Craig J, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. Optom Vis Sci 1997;74(1):8-13.
25. Peng C-C, Cerretani C, Braun RJ, Radke CJ. Evaporation-driven instability of the precorneal tear film. Adv Colloid Interface Sci 2013;206:1-15.
26. Liu H, Begley C, Chen M, et al. A link between tear instability and hyperosmolarity in dry eye. Invest Ophthalmol Vis Sci 2009;50(8):3671-3679.
27. Lemp M a, Bron AJ, Baudouin C, et al. Tear osmolarity in the diagnosis and management of dry eye disease. Am J Ophthalmol 2011;151(5):792-798.e1.
28. King-Smith PE, Nichols JJ, Nichols KK, Fink B a, Braun RJ. Contributions of evaporation and other mechanisms to tear film thinning and break-up. Optom Vis Sci 2008;85(8):623-630.
29. Rosenthal P, Baran I, Jacobs DS. Corneal pain without stain: is it real? Ocul Surf 2009;7(1):28-40.
30. Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol Dis 2001;8(1):1-10.
31. Tuisku IS, Konttinen YT, Konttinen LM, Tervo TM. Alterations in corneal sensitivity and nerve morphology in patients with primary Sjögren's syndrome. Exp Eye Res 2008;86(6):879-885.
32. Braun RJ, Gewecke NR, Begley CG, Ewen King-Smith P, Siddique JI. A model for tear film thinning with osmolarity and fluorescein. Investig Ophthalmol Vis Sci 2014;55(2):1133-1142.
33. Parra A, Gonzalez-Gonzalez O, Gallar J, Belmonte C. Tear fluid hyperosmolality increases nerve impulse activity of cold thermoreceptor endings of the cornea. Pain 2014;155(8):1481-1491.
34. Guillon M, Maissa C. Contact lens wear affects tear film evaporation. Eye Contact Lens 2008;34(6):326-330.
35. Fornasiero F, Prausnitz JM, Radke CJ. Post-lens tear-film depletion due to evaporative dehydration of a soft contact lens. J Memb Sci 2006;275(1-2):229-243.
36. Knop E, Knop N, Millar T, Obata H, Sullivan D a. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy,

physiology, and pathophysiology of the meibomian gland. Invest Ophthalmol Vis Sci 2011;52(4):1938-1978.
37. Pflugfelder SC. Tear dysfunction and the cornea: LXVIII Edward Jackson Memorial Lecture. Am J Ophthalmol 2011;152(6):900-909.e1.
38. Acosta MC, Gallar J, Belmonte C. The influence of eye solutions on blinking and ocular comfort at rest and during work at video display terminals. Exp Eye Res 1999;68(6):663-669.
39. Fox S, Rainie L. Part 1: How the internet has woven itself into American life. Pew Res Cent 2014. http://www.pewinternet.org/2014/02/27/part-1-how-the-internet-has-woven-itself-into-american-life/.
40. Nielson. Total Audience Report. 2014.
41. Blehm C, Vishnu S, Khattak A, Mitra S, Yee RW. Computer Vision Syndrome: A Review. Surv Ophthalmol 2005;50(3):253-262.
42. Rosenfield M, Jahan S, Nunez K, Chan K. Cognitive demand, digital screens and blink rate. Comput Human Behav 2015;51:403-406.
43. Patel S, Henderson R, Bradley L, Galloway B, Hunter L. Effect of visual display unit use on blink rate and tear stability. Optom Vis Sci 1991;68(11):888-892.
44. Chu C, Rosenfield M, Portello JK, Benzoni J a, Collier JD. A comparison of symptoms after viewing text on a computer screen and hardcopy. Ophthalmic Physiol Opt 2011;31(1):29-32.
45. Karpecki PM. Why Dry Eye Trials Often Fail. Rev Optom 2013:50-56.
46. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. Cornea 2004;23(8):762-770.
47. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. Invest Ophthalmol Vis Sci 2011;52(4):2006-2049.
48. Cuevas M, González-García MJ, Castellanos E, et al. Correlations among symptoms, signs, and clinical tests in evaporative-type dry eye disease caused by Meibomian gland dysfunction (MGD). Curr Eye Res 2012;37(10):855-863.
49. Rolando M, Refojo MF. Tear evaporimeter for measuring water evaporation rate from the tear film under controlled conditions in humans. Exp Eye Res 1983;36(1):25-33.
50. Arciniega JC, Wojtowicz JC, Mohamed EM, McCulley JP. Changes in the evaporation rate of tear film after digital expression of meibomian glands in patients with and without dry eye. Cornea 2011;30(8):843-847.
51. Kimball SH, King-Smith PE, Nichols JJ. Evidence for the major contribution of evaporation to tear film thinning between blinks. Invest Ophthalmol Vis Sci 2010;51(12):6294-6297.
52. Mapstone R. Normal thermal patterns in cornea and periorbital skin. Br J Ophthalmol 1968;52(11):818-827.
53. Mapstone R. Determinants of Corneal Temperature. Br J Ophthalmol 1968;52:729-740.
54. Tan J-H, Ng EYK, Acharya UR. Evaluation of tear evaporation from ocular surface by functional infrared thermography. Med Phys 2010;37(11):6022-6034.
55. Tan J-H, Ng EYK, Rajendra Acharya U, Chee C. Infrared thermography on ocular surface temperature: A review. Infrared Phys Technol 2009;52(4):97-108.

56. Craig JP, Singh I, Tomlinson A, Morgan PB. The role of tear physiology in ocular surface temperature. Eye 2000;14:635-641.
57. Morgan P, Tullo A, Efron N. Infrared thermography of the tear film in dry eye. Eye 1995;9:615-618.
58. Morgan PB, Tull AB, Efron N. Ocular surface cooling in dry eye—a pilot study. J Br Contact Lens 1996;19(1):7-10.
59. Girard F, Antoni M, Sefiane K. Infrared thermography investigation of an evaporating sessile water droplet on heated substrates. Langmuir 2010;26(7):4576-4580.
60. Miller W, Millis E. Estimating Evaporation From Utah's Great Salt Lake Using Thermal Infrared Satellite Imagery. Water Resour Bull 1990;25(3):541-550.
61. Kalma JD, Jupp DLB. Estimating evaporation from pasture using infrared thermometry: evaluation of a one-layer resistance model. Agric For Meteorol 1990;51(3-4):223-246.
62. Nichols JJ, King-Smith PE, Hinel E a, Thangavelu M, Nichols KK. The use of fluorescent quenching in studying the contribution of evaporation to tear thinning. Invest Ophthalmol Vis Sci 2012;53(9):5426-5432.
63. Giraldez MJ, Naroo S a, Resua CG. A preliminary investigation into the relationship between ocular surface temperature and lipid layer thickness. Cont Lens Anterior Eye 2009;32(4):177-180; quiz 193, 195.
64. Purslow C, Wolffsohn J. The Relation between Physical Properties of the Anterior Eye and Ocular Surface Temperature. Optom Vis Sci 2007;84(3):197-201.
65. Kamao T, Yamaguchi M, Kawasaki S, Mizoue S, Shiraishi A, Ohashi Y. Screening for dry eye with newly developed ocular surface thermographer. Am J Ophthalmol 2011;151(5):782-791.e1.
66. Su T-Y, Chang S-W, Yang C-J, Chiang HK. Direct observation and validation of fluorescein tear film break-up patterns by using a dual thermal-fluorescent imaging system. Biomed Opt Express 2014;5(8):2614-2619.
67. Petznick A, Tan JH, Boo SK, Lee SY, Acharya UR, Tong L. Repeatability of a new method for measuring tear evaporation rates. Optom Vis Sci 2013;90(4):366-371.
68. Purslow C, Wolffsohn JS. Ocular Surface Temperature. Eye Contact Lens Sci Clin Pract 2005;31(3):117-123.
69. Kottaiyan R, Yoon G, Wang Q, Yadav R, Zavislan JM, Aquavella J V. Integrated multimodal metrology for objective and noninvasive tear evaluation. Ocul Surf 2012;10(1):43-50.
70. Hay EM, Thomas E, Pal B, Hajeer a, Chambers H, Silman a J. Weak association between subjective symptoms or and objective testing for dry eyes and dry mouth: results from a population based study. Ann Rheum Dis 1998;57(1):20-24.
71. Hirata H, Fried N, Oshinsky ML. Quantitative characterization reveals three types of dry-sensitive corneal afferents: pattern of discharge, receptive field, and thermal and chemical sensitivity. J Neurophysiol 2012;108(9):2481-2493.
72. Hirata H, Oshinsky ML. Ocular dryness excites two classes of corneal afferent neurons implicated in basal tearing in rats: involvement of transient receptor potential channels. J Neurophysiol 2012;107(4):1199-1209.
73. Belmonte C, Brock J a, Viana F. Converting cold into pain. Exp Brain Res 2009;196(1):13-30.

74. Hirata H, Meng ID. Cold-sensitive corneal afferents respond to a variety of ocular stimuli central to tear production: implications for dry eye disease. Invest Ophthalmol Vis Sci 2010;51(8):3969-3976.
75. Hirata H, Rosenblatt MI. Hyperosmolar Tears Enhance Cooling Sensitivity of the Corneal Nerves in Rats: Possible Neural Basis for Cold-Induced Dry Eye Pain. Invest Ophthalmol Vis Sci 2014;55:5821-5833.
76. Begley C, Simpson T, Liu H, et al. Quantitative analysis of tear film fluorescence and discomfort during tear film instability and thinning. Investig Ophthalmol Vis Sci 2013.
77. Rosenthal P, Borsook D. The corneal pain system. Part I: the missing piece of the dry eye puzzle. Ocul Surf 2012;10(1):2-14.
78. Niederer RL, McGhee CNJ. Clinical in vivo confocal microscopy of the human cornea in health and disease. Prog Retin Eye Res 2010;29(1):30-58.
79. Wise RJ, Sobel RK, Allen RC. Meibography: A review of techniques and technologies. Saudi J Ophthalmol Off J Saudi Ophthalmol Soc 2012;26(4):349-356.
80. Gatchel RJ, Peng YB, Peters ML, Fuchs PN, Turk DC. The biopsychosocial approach to chronic pain: scientific advances and future directions. Psychol Bull 2007;133(4):581-624.
81. Villemure C, Bushnell MC. Cognitive modulation of pain: how do attention and emotion influence pain processing? Pain 2002;95(3):195-199.
82. Linton SJ, Shaw WS. Impact of psychological factors in the experience of pain. Phys Ther 2011;91(5):700-711.
83. Greenspan JD, Craft RM, LeResche L, et al. Studying sex and gender differences in pain and analgesia: a consensus report. Pain 2007;132 Suppl :S26-S45.
84. Kim H, Neubert JK, San Miguel A, et al. Genetic influence on variability in human acute experimental pain sensitivity associated with gender, ethnicity and psychological temperament. Pain 2004;109(3):488-496.
85. Nielsen CS, Staud R, Price DD. Individual differences in pain sensitivity: measurement, causation, and consequences. J Pain 2009;10(3):231-237.
86. Fillingim RB. Individual differences in pain responses. Curr Rheumatol Rep 2005;7(5):342-347.
87. Definition of Discomfort. Merriam Webster Dict 2013. http://www.merriam-webster.com/dictionary/discomfort.
88. Green CR, Anderson KO, Baker T a, et al. The unequal burden of pain: confronting racial and ethnic disparities in pain. Pain Med 2003;4(3):277-294.
89. Kil HK, Kim WO, Chung WY, Kim GH, Seo H, Hong J-Y. Preoperative anxiety and pain sensitivity are independent predictors of propofol and sevoflurane requirements in general anaesthesia. Br J Anaesth 2012;108(1):119-125.
90. Greenwald HP. Interethnic differences in pain perception. Pain 1991;44(2):157-163.
91. Chan MYP, Hamamura T, Janschewitz K. Ethnic differences in physical pain sensitivity: Role of acculturation. Pain 2013;154(1):119-123.
92. Ruscheweyh R, Marziniak M, Stumpenhorst F, Reinholz J, Knecht S. Pain sensitivity can be assessed by self-rating: Development and validation of the Pain Sensitivity Questionnaire. Pain 2009;146(1-2):65-74.

93. Tan E-C, Lim Y, Teo Y-Y, Goh R, Law H-Y, Sia AT. Ethnic differences in pain perception and patient-controlled analgesia usage for postoperative pain. J Pain 2008;9(9):849-855.
94. Rahim-williams B, Fillingim RB. A Quantitative Review of Ethnic Group Differences in Experimental Pain Response : Do Biology , Psychology , and Culture Matter ? Pain Med 2012;13(4):522-540.
95. Belmonte C, Garcia-hirschfeld J, Gallar J, Neurociencias I De, Fisiologia D De, Alicante U De. Neurobiology of Ocular Pain. Prog Retin Eye Res 1996;9462(96):118-149.
96. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. Neuron 2007;55(3):377-391.
97. Flor H. Cortical reorganisation and chronic pain: implications for rehabilitation. J Rehabil Med 2003;35(4):66-72.
98. Bushnell MC, Ceko M, Low L a. Cognitive and emotional control of pain and its disruption in chronic pain. Nat Rev Neurosci 2013;14(7):502-511.
99. Ruscheweyh R, Verneuer B, Dany K, et al. Validation of the Pain Sensitivity Questionnaire in chronic pain patients. Pain 2012;153(6):1210-1218.
100. Granot M. Can we predict persistent postoperative pain by testing preoperative experimental pain? Curr Opin Anaesthesiol 2009;22(3):425-430.
101. Chapman CR, Donaldson GW, Davis JJ, Bradshaw DH. Improving individual measurement of postoperative pain: the pain trajectory. J Pain 2011;12(2):257-262.
102. Edwards RR. Individual differences in endogenous pain modulation as a risk factor for chronic pain. Neurology 2005;65(3):437-443.
103. Mobilio N, Gremigni P, Pramstraller M, Vecchiatini R, Calura G, Catapano S. Explaining pain after lower third molar extraction by preoperative pain assessment. J Oral Maxillofac Surg 2011;69(11):2731-2738.
104. Kim H-J, Yeom JS, Lee JW, et al. The Influence of Pain Sensitivity on the Treatment Outcome of Transforaminal Epidural Steroid Injection in Patients with Lumbar Spinal Stenosis. Pain Pract 2013;14(5):405-412.
105. Kim H-J, Ruscheweyh R, Yeo J-H, et al. Translation, Cross-Cultural Adaptation, and Validity of the Korean Version of the Pain Sensitivity Questionnaire in Chronic Pain Patients. Pain Pract 2013:1-7.
106. Kim H-J, Suh B-G, Lee D-B, et al. Gender difference of symptom severity in lumbar spinal stenosis: role of pain sensitivity. Pain Physician 2013;16(6):E715-E723.
107. Kim H-J, Suh B-G, Lee D-B, et al. The influence of pain sensitivity on the symptom severity in patients with lumbar spinal stenosis. Pain Physician 2013;16(2):135-144.
108. Riley C, Young G, Chalmers R. Prevalence of ocular surface symptoms, signs, and uncomfortable hours of wear in contact lens wearers: the effect of refitting with daily-wear silicone hydrogel lenses (senofilcon a). Eye Contact Lens 2006;32(6):281-286.
109. Tan J-H, Ng EYK, Acharya UR. Evaluation of tear evaporation from ocular surface by functional infrared thermography. Med Phys 2010;37(11):6022-6034.
110. Klamann MKJ, Maier A-KB, Gonnermann J, Klein JP, Pleyer U. Measurement of

dynamic ocular surface temperature in healthy subjects using a new thermography device. Curr Eye Res 2012;37(8):678-683.
111. Purslow C, Wolffsohn JS, Santodomingo-Rubido J. The effect of contact lens wear on dynamic ocular surface temperature. Cont Lens Anterior Eye 2005;28(1):29-36.
112. King-Smith PE, Reuter KS, Braun RJ, Nichols JJ, Nichols KK. Tear film breakup and structure studied by simultaneous video recording of fluorescence and tear film lipid layer images. Invest Ophthalmol Vis Sci 2013;54(7):4900-4909.
113. Begley C, Himebaugh N. Tear Breakup Dynamics: A Technique for Quantifying Tear Film Instability. Optom Vis … 2006;83(1):15-21.
114. Freeman R, Fatt I. Environmental influences on ocular temperature. Invest Ophthalmol Vis Sci 1973;12(8):596-602.
115. Biondi F, Dornbusch PT, Sampaio M, Montiani-Ferreira F. Infrared ocular thermography in dogs with and without keratoconjunctivitis sicca. Vet Ophthalmol 2013:1-7.
116. Bron a. J, Tiffany JM. The Contribution of Meibomian Disease to Dry Eye. Ocul Surf 2004;2(2):149-164.
117. Johnson ME, Murphy PJ. The Effect of instilled fluorescein solution volume on the values and repeatability of TBUT measurements. Cornea 2005;24(7):811-817.
118. Nichols J, Sinnott L. Tear Film, Contact Lens, and Patient-Related Factors Associated with Contact Lens – Related Dry Eye. Invest Ophthalmol Vis Sci 2006;47(4):1319-1328.
119. Nichols KK, Foulks GN, Bron AJ, et al. The international workshop on meibomian gland dysfunction: executive summary. Invest Ophthalmol Vis Sci 2011;52(4):1922-1929.
120. Gaffney E a., Tiffany JM, Yokoi N, Bron a. J. A mass and solute balance model for tear volume and osmolarity in the normal and the dry eye. Prog Retin Eye Res 2010;29(1):59-78.
121. King-Smith PE, Ramamoorthy P, Braun RJ, Nichols JJ. Tear Film Images and Breakup Analyzed Using Fluorescent Quenching. Invest Ophthalmol Vis Sci 2013.
122. Li WY, Graham AD, Lin MC. Ocular Surface Cooling Corresponds to Tear Film Thinning and BreakupNo Title. Optom Vis Sci 2015;In Press(9):248-256.
123. Tan LL, Sanjay S, Morgan PB. Repeatability of infrared ocular thermography in assessing healthy and dry eyes. Contact Lens Anterior Eye 2016;(2015):1-9.
124. Kessel L, Johnson L, Arvidsson H, Larsen M. The relationship between body and ambient temperature and corneal temperature. Invest Ophthalmol Vis Sci 2010;51(12):6593-6597.
125. Fleiss JL. *Statistical Methods for Rates and Proportions. 2nd Ed.*; 1981.
126. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1(fig 1):307-310.
127. Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. Cornea 2004;23(3):272-285.
128. Abreau K, Callan C, Kottaiyan R, et al. Temperatures of the Ocular Surface, Lid, and Periorbital Regions of Sjögren's, Evaporative, and Aqueous-Deficient Dry Eyes Relative to Normals. Ocul Surf 2016;14(1):64-73.
129. Li L, Braun RJ. A model for the human tear film with heating from within the eye.

Phys Fluids 2012;24(6):062103.
130. Deng Q, Braun RJ, Driscoll T a. Heat transfer and tear film dynamics over multiple blink cycles. Phys Fluids 2014;26:071901.
131. Fujishima H, Toda I, Yamada M, Sato N, Tsubota K. Corneal temperature in patients with dry eye evaluated by infrared radiation thermometry. Br J Ophthalmol 1996;80(1):29-32.
132. Scott J a. A finite element model of heat transport in the human eye. Phys Med Biol 1988;33(2):227-241.
133. Pult H, Riede-Pult BH, Murphy PJ. A New Perspective on Spontaneous Blinks. Ophthalmology 2013:1-6.
134. Yan Z, Hu L, Chen H, Lu F. Computer Vision Syndrome: A widely spreading but largely unknown epidemic among computer users. Comput Human Behav 2008;24(5):2026-2042.
135. Cardona G, García C, Serés C, Vilaseca M, Gispets J. Blink rate, blink amplitude, and tear film integrity during dynamic visual display terminal tasks. Curr Eye Res 2011;36(3):190-197.
136. Hayes JR, Sheedy JE, Stelmack J a, Heaney C a. Computer use, symptoms, and quality of life. Optom Vis Sci 2007;84(8):738-744.
137. George S. Psychologic influence on experimental pain sensitivity and clinical pain intensity for patients with shoulder pain. J Pain 2009;10(3):293-299.
138. Nederhof a J. Methods of coping with social desirability bias: a reivew. Euro J Soc Psych 1985;15(April 1984):263-280.
139. Rushworth G. Observations on Blink Reflexes. J Neurol Neurosurg Psychiatry 1962;25(93):93-108.
140. Kawashima M, Tsubota K. Tear lipid layer deficiency associated with incomplete blinking: A case report. BMC Ophthalmol 2013;13(1):34.
141. Wan T, Jin X, Lin L, Xu Y, Zhao Y. Incomplete Blinking May Attribute to the Development of Meibomian Gland Dysfunction. Curr Eye Res 2015;3683(September):1-7.
142. Butovich I. Lipidomics of human Meibomian gland secretions: Chemistry, biophysics, and physiological role of Meibomian lipids. Prog Lipid Res 2011;50(3):278-301.
143. Korb DR, Baron DF, Herman JP, et al. Tear film lipid layer thickness as a function of blinking. Cornea 1994;13(4):354-359.
144. Butovich I, Millar TJ, Ham BM. Understanding and analyzing meibomian lipids--a review. Curr Eye Res 2008;33(5):405-420.
145. King-Smith PE, Bailey MD, Braun RJ. Four characteristics and a model of an effective tear film lipid layer (TFLL). Ocul Surf 2013;11(4):236-245.
146. Rosenfeld L, Cerretani C, Leiske DL, Toney MF, Radke CJ, Fuller GG. Structural and Rheological Properties of Meibomian Lipid. 2013.
147. Viitala T, Kulovesi P, Telenius J, et al. Molecular Organization of the Tear Fluid Lipid Layer. Biophys J 2010;99(October):2559-2567.
148. Wizert A, Iskander DR, Cwiklik L. Organization of Lipids in the Tear Film : A Molecular-Level View. PLoS One 2014;9(3):1-10.
149. Nichols JJ, Willcox MDP, Bron AJ, et al. The TFOS International Workshop on Contact Lens Discomfort: executive summary. Invest Ophthalmol Vis Sci

2013;54(11):TFOS7-TFOS13.
150. Young G, Chalmers R, Napier L, Kern J, Hunt C, Dumbleton K. Soft contact lens-related dryness with and without clinical signs. Optom Vis Sci 2012;89(8):1125-1132.
151. Peters ML. Emotional and Cognitive influences on pain experience. Mod Trends Pharmacopsychiatry 2015;30(1):138-152.
152. Hart RP, Wade JB, Martelli MF. Cognitive impairment in patients with chronic pain: the significance of stress. Curr Pain Headache Rep 2003;7(2):116-126.
153. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: A review of clinical and preclinical research. Prog Neurobiol 2011;93(3):385-404.
154. Baudouin C, Aragona P, Van Setten G, et al. Diagnosing the severity of dry eye: a clear and practical algorithm. Br J Ophthalmol 2014;98(9):1168-1176.
155. Truong TN, Graham AD, Lin MC. Factors in Contact Lens Symptoms : Evidence from a Multistudy Database. 2014;91(2):133-141.
156. Craig JP, Willcox MDP, Argüeso P, et al. The TFOS International Workshop on Contact Lens Discomfort: Report of the contact lens interactions with the tear film subcommittee. Investig Ophthalmol Vis Sci 2013;54(11):TFOS123-TFOS156.
157. Jones L, Brennan N a., González-Méijome J, et al. The TFOS International Workshop on Contact Lens Discomfort: Report of the contact lens materials, design, and care subcommittee. Investig Ophthalmol Vis Sci 2013;54(11):TFOS37-TFOS70.
158. Tran N, Graham AD, Lin MC. Ethnic differences in dry eye symptoms: effects of corneal staining and length of contact lens wear. Cont Lens Anterior Eye 2013;36(6):281-288.
159. Vehof J, Kozareva D, Hysi PG, et al. Relationship Between Dry Eye Symptoms and Pain Sensitivity. JAMA Ophthalmol 2013:1-5.
160. Hsieh AY, Tripp D a, Ji L-J, Sullivan MJL. Comparisons of catastrophizing, pain attitudes, and cold-pressor pain experience between Chinese and European Canadian young adults. J Pain 2010;11(11):1187-1194.
161. Svitova TF, Lin MC. Racial variations in interfacial behavior of lipids extracted from worn soft contact lenses. Optom Vis Sci 2013;90(12):1361-1369.
162. Lin MC, French HM, Graham AD, Sanders TL. Effects of daily irrigation on corneal epithelial permeability and adverse events with silicone hydrogel contact lens continuous wear. Invest Ophthalmol Vis Sci 2014;55(2):776-783.
163. Lin MC, Yuen J, Graham AD. Contact Lens Care Solutions. Eye Contact Lens Sci Clin Pract 2014;40(4):191-199.
164. Lin MC, Yeh TN, Graham AD, et al. Ocular surface health during 30-day continuous wear: rigid gas-permeable versus silicone hydrogel hyper-O2 transmitted contact lenses. Invest Ophthalmol Vis Sci 2011;52(6):3530-3538.
165. Lin MC, Chen YQ, Polse K a. The effects of ocular and lens parameters on the postlens tear thickness. Eye Contact Lens 2003;29(1 Suppl):S33-S36 - discussion S57-S59 - S192-S194.
166. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. J Clin Epidemiol 2010;63(8):834-840.
167. Miller KL, Walt JG, Mink DR, et al. Minimal Clinically Important Difference for the

Ocular Surface Disease Index. 2014;128(1):94-101.
168. Rowell LN, Mechlin B, Ji E, Addamo M, Girdler SS. Asians differ from non-Hispanic Whites in experimental pain sensitivity. Eur J Pain 2011;15(7):764-771.
169. Lu Q, Tsao J. Multi-Ethnic Differences in Responses to Laboratory Pain Stimuli among Children. Heal Pschology 2013;32(8):905-914.
170. Maïssa C, Guillon M. Tear film dynamics and lipid layer characteristics--effect of age and gender. Cont Lens Anterior Eye 2010;33(4):176-182.
171. Nien CJ, Massei S, Lin G, et al. Effects of age and dysfunction on human meibomian glands. Arch Ophthalmol 2011;129(4):462-469.
172. Pult H, Purslow C, Berry M, Murphy PJ. Clinical tests for successful contact lens wear: relationship and predictive potential. Optom Vis Sci 2008;85(10):E924-E929.
173. Shiraishi A, Yamaguchi M, Ohashi Y. Prevalence of Upper- and Lower-Lid-Wiper Epitheliopathy in Contact Lens Wearers and Non-wearers. Eye Contact Lens Sci Clin Pract 2014;40(4):220-224.
174. Su TY, Ho WT, Chang SW, Chiang HK. Thermographic evaluation of tear film break-up time to study tear film stability. Int J Therm Sci 2016;99:36-40.
175. Tsubota K, Yamada M. Tear evaporation from the ocular surface. Invest Ophthalmol Vis Sci 1992;33(10):2942-2950.
176. Kojima T, Matsumoto Y, Ibrahim OM a, et al. Effect of controlled adverse chamber environment exposure on tear functions in silicon hydrogel and hydrogel soft contact lens wearers. Investig Ophthalmol Vis Sci 2011;52(12):8811-8817.
177. McCann LC, Tomlinson A, Pearce EI, Diaper C. Tear and meibomian gland function in blepharitis and normals. Eye Contact Lens 2009;35(4):203-208.
178. Azharuddin M, Bera SK, Datta H, Dasgupta AK. Thermal fluctuation based study of aqueous deficient dry eyes by non-invasive thermal imaging. Exp Eye Res 2014;120C:97-102.
179. Mishima S, Maurice DM. The oily layer of the tear film and evaporation from the corneal surface. Exp Eye Res 1961;1(Sep):39-45.
180. Tan JH, Ng EYK, Acharya UR. The Effect of Tear Film on Ocular Surface Temperature: A Thermodynamic Study. J Heat Transfer 2013;135(5):054505.
181. Purslow C. Evaluation of the ocular tolerance of a novel eyelid-warming device used for meibomian gland dysfunction. Cont Lens Anterior Eye 2013;36(5):226-231.
182. Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. Nat Med 2010;16(11):1248-1257.
183. Parra A, Madrid R, Echevarria D, et al. Ocular surface wetness is regulated by TRPM8-dependent cold thermoreceptors of the cornea. Nat Med 2010;16(12):1396-1399.
184. Hondoh A, Ishida Y, Ugawa S, et al. Distinct expression of cold receptors (TRPM8 and TRPA1) in the rat nodose-petrosal ganglion complex. Brain Res 2010;1319:60-69.
185. Tracey I. Nociceptive processing in the human brain. Curr Opin Neurobiol 2005;15(4):478-487.
186. Schmelz M. Translating nociceptive processing into human pain models. Exp Brain Res 2009;196(1):173-178.