Contemporary Diagnosis and Management of Dry Eye

by

William Ngo

A thesis
presented to the University of Waterloo
in fulfilment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Vision Science

Waterloo, Ontario, Canada, 2016

Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Statement of Contributions

I would like to acknowledge the names of my co-authors who contributed to this thesis:

- Dr. Lyndon Jones, PhD, FCOptom
- Dr. Sruthi Srinivasan, PhD, BS Optom
- Dr. Marc Schulze, PhD, Dipl Ing (AO)
- Dr. Diane Houtman, OD, MBA
- Dr. Barbara Caffery, OD, PhD

Abstract

Introduction

Dry eye (DE) disease is characterized by symptoms including, but not limited to, ocular stinging, burning, and tearing. The symptoms can range from being mildly irritating, to severely debilitating and negatively impacting quality of life. The impairment of the tear film, lacrimal functional unit, and meibomian glands (MGs) causes desiccation of the ocular surface, which in turn promotes and exacerbates inflammation. Dry eye is a multifactorial disease and the many causative factors can be completely exclusive from each other. This necessitates that the management of DE be multi-faceted. Dry eye disease affects millions of people around the world and this number will increase as the elderly population rise over the next few decades. However, emerging technologies are allowing clinicians and scientists to constantly discover new ways to diagnose and manage various aspects of DE. The global aim of this thesis was to evaluate some of the contemporary methods used in the diagnosis and management of DE disease.

The specific aims of each chapter were as follows:

- Chapter 3: To determine whether an experimental spectral domain ultra-long optical coherence tomographer (UL-OCT) can image MGs, and to compare its performance to the Heidelberg Retina Tomograph 3 (HRT3) with Rostock Cornea Module (RCM) *in vivo* laser scanning confocal microscope (Heidelberg Engineering GmbH, Heidelberg, Germany) and the Keratograph 5M (K5M) (OCULUS, Wetzlar, Germany).
- Chapter 4: To determine the inter- and intra-observer repeatability in using the Keratograph 4 (K4) and K5M to grade MG dropout using meibography grading scales.
- Chapter 5: To quantify the association of DE diagnostic tests to DE symptoms in an age-matched female cohort.
- Chapter 6: To determine the effectiveness of an eyelid warming device in the management of MG dysfunction (MGD).

- Chapter 7: To evaluate the effect of lid debridement-scaling (LDS) on DE signs and symptoms in individuals with Sjögren's syndrome (SS).
- Chapter 8: To determine the combined effect of a lubricant eye drop, lid hygiene, and omega 3 fatty acids on DE signs and symptoms.

Methods

- Chapter 3: The superior eyelids of 12 participants were everted and imaged using the UL-OCT. The inferior and superior eyelids were then everted and imaged using the K5M. Finally, the inferior eyelids were everted and imaged with the HRT3/RCM.
- Chapter 4: The inferior and superior eyelids of 40 participants were imaged 3 times each on both the K4 and K5M. The images were split into one training and two study sets; the latter were graded (4-point meibography scale) by two observers on two separate occasions (24hrs apart) to examine repeatability. Semi-objective grading of MG dropout was conducted using ImageJ on K4 and K5M images. A 7-point meibography scale was used to grade a separate set of K5M images.
- Chapter 5: Twenty females symptomatic of DE (Ocular Surface Disease Index, OSDI, ≥ 13) were age-matched to 20 asymptomatic females (OSDI < 13). Non-invasive tear breakup time (NIBUT), ocular staining, meibum quality, number of obstructed glands, lid wiper epitheliopathy (LWE), Line of Marx (LOM) placement, eyelid margin score, Schirmer's test, meibography, and visual acuity were compared between the two groups.
- Chapter 6: This was a prospective, randomized, controlled, single-masked, bilateral eye study that enrolled 29 participants. Participants were randomized into either the EyeBag group or the control group. Participants in the EyeBag group were instructed to use the EyeBag 10 minutes 2x/day, and the control group remained on their own DE treatment regimen (if applicable). All participants were seen at baseline, 2 weeks and 4 weeks. At 4 weeks, participants in the EyeBag group were asked to stop using the EyeBag. All participants were seen again at the 8 week mark. Primary

outcomes were the OSDI, Current Symptoms Questionnaire (CSQ), MG score (MGS), and non-invasive tear break-up time (NIBUT).

- Chapter 7: This prospective randomized controlled study enrolled 14 female participants with SS. Seven participants were randomized into the treatment group, where they were selected to receive LDS, the remainder did not receive LDS and served as controls. Lid debridement-scaling was conducted using a stainless steel golf club spud (Hilco Wilson Ophthalmics, Plainville, MA) on both the upper and lower eyelids of both eyes. Outcome variables were assessed prior to LDS and again 1 month later. The outcome variables were the OSDI, Symptoms iN Assessment of Dry Eye (SANDE) visual analogue scores, Sjögren's International Collaborative Clinical Alliance Ocular Staining Score (SICCA OSS), fluorescein tear breakup time (FLBUT), MGS, MG yielding liquid secretions score (MGYLS), and LOM position.
- Chapter 8: This prospective study enrolled 28 DE participants. Participants were instructed to use the TheraTears® Lubricant Eye Drops at least 2-4x a day, TheraTears® SteriLid 1-2x a day, and TheraTears® Nutrition 3 gel caps once a day. Participants were followed up at baseline, 1 month and 3 months. Outcome variables were the OSDI, SANDE, NIBUT, osmolarity, number of MGs blocked (#MG blocked), meibum quality, eyelid margin features, Schirmer's test, tear film lipid layer thickness (LLT), meniscus height, corneal and conjunctival staining.

Results

- Chapter 3: All twelve participants (7 female, 5 male) completed the study. The only instrument that was able to successfully image MGs was the K5M.
- Chapter 4: When using the 4-point scale, inter-observer mean difference (MD) was 0.08±0.55 on day 1 and 0.13±0.50 on day 2, and the concordance correlation coefficient (CCC) was 0.79 and 0.81 on days 1 and 2 respectively. Intra-observer MD was 0.04±0.54, CCC=0.79 for observer 1, and -0.09±0.60, CCC=0.74 for observer 2. For the 7-point scale, inter-observer MD was 0.05±0.45, CCC=0.89 on day 1 and 0.01±0.41, CCC=0.91 on day 2. Intra-observer MD was -0.10±0.35,

CCC=0.93 for observer 1 and -0.06±0.30, CCC=0.95 for observer 2. Percentage dropout measured between the K4 and K5M images showed lack of agreement, with only a 21.8% coefficient of repeatability.

- Chapter 5: Twenty participant-pairs completed the study. The age (median/interquartile range(IQR)) of the symptomatic group was (60/15) and the asymptomatic group was (62/15). The diagnostic tests (median/IQR, p-value) that were significantly different between the symptomatic group vs. the asymptomatic group were OSDI (35.4/35.4 vs. 3.1/6.7, $p < 0.01$), NIBUT (2.1s/0.7s vs. 3.0s/3.0s, $p = 0.01$), meibum quality (3.0/0.0 grade units vs. 2.0/1.0 grade units, $p < 0.01$), number of obstructed glands (7.0/2.0 glands vs. 5.0/4.8 glands, $p < 0.01$), and ocular staining (5.5/3.8 grade units vs. 0.5/1.0 grade units, $p < 0.01$). The diagnostic tests (area under curve (AUC), odds ratio (OR)) that were most strongly associated with DE symptoms were ocular staining (0.93, 5.0), number of glands obstructed (0.79, 2.6), meibum quality (0.76, 2.4), and NIBUT (0.74, 3.2) (all $p < 0.05$). There was no significant difference between the two groups for the other DE diagnostic tests (all $p > 0.05$), and similarly, no significant association to DE symptoms (all $p > 0.05$).
- Chapter 6: Twenty five participants completed the study (mean age 38±15 years, 7 male). There was a significant change in OSDI over time for the EyeBag group (mean values±SD, baseline: 39.1±12.5, week 2: 26.8±11.2, week 4: 26.6±26.6, week 8: 27.7±14.6; $p<0.05$), but no significant change in the control group. There was a significant improvement in symptoms immediately after conducting the EyeBag based on at-home CSQ scores (Δ=-5.0 points, $p<0.05$), but no significant change in the control group. There was no significant change in MGS and NIBUT over time for either group.
- Chapter 7: There were 13 participants that completed the study. Data from the right eye only were analyzed. For the control group (n=6, mean age=62±12), the pre LDS, post LDS, and significance level (pre-mean±SD, post-mean±SD; p-value) were: OSDI (58.3±22.1, 48.3±29.0; p=0.051), SANDE (77.4±22.1, 89.6±32.6; p=0.20), SICCA OSS (7.0±4.5, 8.2±3.5; p=0.25), MGS (1.3±1.5,

1.0±0.9; p=0.75), MGYLS (0.3±0.5, 0.0±0.0; p=0.50), FLBUT (2.99 ±1.54, 2.85±1.79; p=0.63), LOM (2.0±0.0, 2.0±0.0, p=n/a). For the treatment group (n=7, mean age=58±8), the pre LDS, post LDS, and significance level were: OSDI (63.2±13.3, 46.9±19.4; p=0.04), SANDE (72.6±17.1, 77.0±28.0; p=0.54), SICCA OSS (6.6±2.9, 5.0±3.9; p=0.02), MGS (1.0±1.2, 3.1±1.7; p=0.01), MGYLS (0.0±0.0, 0.6±1.0; p=0.50), FLBUT (3.13±0.81, 3.45±1.03; p=0.53), LOM (0.9±0.9, 1.0±1.0, p=1.00).

- Chapter 8: There were 20 participants (mean age = 43, from 23 to 66, 17F, 3M) that completed the study. On average, participants used the Lubricant Eye Drop 2.4x/day, the SteriLid 1.1x/day, and the Nutrition 3 gel caps 1x/day. There was a significant change over time ($p<0.05$) for OSDI (-21.2 points), SANDE (-32.4 points), NIBUT (+0.43s), eyelid margin features (-1.1 grade), meibum quality (-1.0 grade), and #MG blocked (-4.0 glands).

Conclusions

- Chapter 3: The UL-OCT was unable to image MGs. The HRT3/RCM imaged structures that resembled dermal rete pegs and papillae. Of the three methods used in this study, the only device that was able to successfully image MGs was the K5M.
- Chapter 4: Observers graded from -1 to +1 grade units between and within themselves for a 4-point scale, 95% of the time. Although the inter- and intra-observer repeatability of the K4 and K5M were very similar, a low level of agreement in percentage dropout between K4 and K5M images suggests that the two instruments cannot be interchanged. Using a finer scale may be beneficial for detecting change over time.
- Chapter 5: The diagnostic tests that were most strongly associated with DE symptoms in older women were ocular staining, meibum quality, number of glands obstructed, and tear film stability.
- Chapter 6: The MGDRx® EyeBag was effective at relieving symptoms in participants with DE, but the effect on MG function and tear stability when used for only 4 weeks was modest.

- Chapter 7: This pilot study showed that LDS improved symptoms, ocular staining and MG function for the group that received LDS. This indicates that LDS can aid in the management of SS DE.
- Chapter 8: After using a combination of TheraTears® Lubricant Eye Drop, SteriLid, and Nutrition, participants experience significant relief in both DE symptoms and signs.

This thesis was able to evaluate and suggest improvements to meibography techniques and grading. In addition, this thesis was also able to evaluate the effectiveness of various contemporary methods for DE treatment. The methods used in this study are clinically accessible and clinicians are free to use these findings and apply them directly to their clinical practice.

Acknowledgements

I would like to sincerely thank my supervisors, Dr. Lyndon Jones and Dr. Sruthi Srinivasan for their mentorship and guidance. Thank you both for not only teaching me the technical skills required for conducting research, but also for your generosity in providing me with many opportunities to further my career. Thank you for always offering help, for sharing your wisdom, and for being my guide into the world of dry eye research.

I am grateful to Dr. Marc Schulze for his generous support. Thank you for your help with the UL-OCT, and for offering your insight with statistics and grading scales.

I would also like to thank Dr. Barbara Caffery. I am grateful to you for allowing me to work alongside you and your fantastic clinical team. I am inspired by your constant dedication to both clinical practice and research.

I am also thankful to Dr. Trefford Simpson and Dr. Natalie Hutchings, for not only teaching me the technical skills for statistics and image analysis, but also for encouraging different perspectives on research. Your constant drive to break out of the comfort zone, learn new things, and acquire new skills have inspired me to do the same.

I wish to thank my external examiners, Drs. Jerry Paugh and Brian Dixon for their contribution in reviewing and providing criticism on my work.

I am grateful to the EyeBag Company, OCULUS Optikgeräte, MetricWire, and Damaris Grau for providing study supplies, instruments, and assistance with study procedures.

I wish to thank Dr. Etty Bitton for hosting my stay at the University of Montreal. Thank you for being an amazing tour guide to Quebec, and for sharing your clinical expertise and involving me in your clinic and dry eye research. In addition, I also want to thank the graduate students at the University of Montreal for welcoming me into their family and making my experience at Montreal an unforgettable one.

I also want to thank the graduate officers (Drs. Vivian Choh, Trefford Simpson, Ben Thompson, Daphne McCulloch), the graduate coordinators (Krista Parsons, Lisa Baxter, Jennifer Cosentino, Stephanie Forsyth), and the fellow members of GIVS for their moral support through this work.

I am also grateful to the members of my extended family, the Centre for Contact Lens Research, for their constant support. Thank you all for helping me with my research in this loving environment, and for constantly reminding me that there is life outside of work.

I wish to thank and acknowledge the generous support of the American Optometric Foundation, the Sjögren's Society of Canada, Canadian Optometric Education Trust Fund, and Dr. Desmond Fonn. Your contributions have been greatly appreciated, this work would not have been possible without your help.

Finally, I am thankful for the support and company of my family and friends. Thank you all for supporting me throughout this wonderful journey.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ACR	American College of Rheumatology
AECG	American-European Consensus Group
ALA	Alpha linolenic acid
AUC	Area under curve
BAK	Benzalkonium chloride
CCC	Concordance correlation coefficient
CCD	Charge-coupled device
CL	Contact lens
CCLR	Centre for Contact Lens Research
CM	Confocal microscopy
CSQ	Current Symptoms Questionnaire
DE	Dry eye
DEWS I	Dry Eye Workshop I
DHA	Docosahexaenoic acid
EEC	Ectrodactyly ectodermal dysplasia
EPA	Eicosapentaenoic acid
FD-OCT	Fourier-domain optical coherence tomographer
FDA	Food and Drug Administration
FLBUT	Fluorescein breakup time
HRT, HRT3	Heidelberg Retina Tomograph / Heidelberg Retina Tomograph 3
HP	Heavily-pigmented
ICD	Inflammatory cell density
ICU	Interferometric colour unit
IDEEL	Impact of Dry Eye on Everyday Life
IFAP	Ichthyosis follicularis-alopecia/photophobia
IR	Infrared
IQR	Interquartile range
K4	OCULUS Keratograph 4

K5M	OCULUS Keratograph 5M
LDS	Lid debridement-scaling
LED	Light-emitting diode
LLT	Lipid layer thickness
LOM	Line of Marx
LP	Lightly-pigmented
LWE	Lid wiper epitheliopathy
MD	Mean difference
MG	Meibomian gland
MGALD	Meibomian gland acinar longest diameter
MGALSD	Meibomian gland acinar shortest diameter
MGAUD	Meibomian gland acinar unit density
MGD	Meibomian gland dysfunction
MGS	Meibomian gland score
MGYLS	Meibomian glands yielding liquid secretions
MMP	Matrix metalloproteinase
NIBUT	Non-invasive tear breakup time
NOMGD	Non-obvious meibomian gland dysfunction
O3FA	Omega 3 fatty acids
O6FA	Omega 6 fatty acids
OCT	Optical coherence tomograph
OR	Odds ratio
OSDI	Ocular Surface Disease Index
PRO	Patient-reported outcome
RCM	Rostock Cornea Module
ROC	Receiver operator characteristics
ROI	Region of interest
SANDE	Symptom Assessment in Dry Eye
SICCA OSS	Sjögren's International Collaborative Clinical Alliance Ocular Staining Score
SPEED	Standard Patient Evaluation of Eye Dryness

SS	Sjögren's syndrome
TMSA	Template Matching and Slice Alignment
UL-OCT	Ultra-long optical coherence tomographer

1 Literature Review

1.1 Prevalence and Risk Factors

1.1.1 Prevalence

The prevalence of dry eye (DE), according to the 2007 Report of the International Dry Eye Workshop (DEWS I), was reported to be between 5.5% to 33.7% of all participants, [1] with this prevalence being drawn from several studies in the US (7.8-14.6%),[2-9] Australia (5.5-16.6%),[10,11] Taiwan (33.7%),[12] and Sumatra (27.5%).[13] Since 2007, prevalence data have emerged from a number of other countries, including Jordan (59%, symptoms only),[14] Japan (31.6%-34.1%),[15,16] South Korea (8.0%),[17,18] Iran (8.7%),[19] and mainland China (17.0%).[20] The data from these studies suggest that Asian populations have the highest prevalence of DE. The methods for determining prevalence ranged from measuring DE symptoms only, to being based on various batteries of DE clinical tests, to using combinations of both symptoms and signs. As a result, prevalence information is dependent on the different methods used to obtain data.

1.1.2 Risk Factors

One of the goals for the 2007 DEWS I report was to describe the risk factors for DE disease. The report found that female sex, older age, hormone replacement therapy, refractive surgery, omega 3 fatty acids (O3FA) to omega 6 fatty acids (O6FA) ratio, refractive surgery, vitamin A deficiency, radiation therapy, bone marrow transplantation, hepatitis C, and various classes of medications were all strong risk factors for DE disease.[1] A number of studies conducted after the 2007 DEWS I report supported these findings.

1.1.2.1 Age

A number studies conducted after DEWS I confirmed aging as a prominent risk factor for DE.[21-23] Aging is involved in mechanisms that increase oxidative stress in tissues.[24-28] Inflammation resulting from oxidative

stress has been found to cause a decline in tear production from the lacrimal gland.[24] Increase in oxidative stress can also be triggered by hyperglycemia,[29-31] making diabetes mellitus a further risk factor.

1.1.2.2 Sex

Females are more likely than males to have DE.[2,5,6,14,17,32] Androgens are sex hormones that upregulate meibomian gland (MG) function, whereas estrogen and progesterone downregulate sebaceous gland and lipid production.[33] The production of androgens decline over time in women,[33,34] however the level of androgens in women remains far less than that of men. This may explain why women are more prone to DE than men.[33] Furthermore, hormone replacement therapy to help relieve menopausal symptoms has also been found to cause DE.[35,36]

1.1.2.3 Systemic Conditions and Medications

Numerous systemic conditions and medications can precipitate and exacerbate DE.[1] For example, the lacrimal gland is often a target in autoimmune diseases such as Sjögren's syndrome (SS),[37] systemic lupus erythematosus,[38] and rheumatoid arthritis.[32,39] Parasympathetic input[40] is involved in regulating lacrimal gland activity and medications that modulate acetylcholine or parasympathetic activity can potentially impact the signal for lacrimal secretion. For this reason, anxiolytics,[41,42] anticholinergics, [42,43] and antihistamines[32,42] can downregulate lacrimal gland function. Medications that manage severe acne vulgaris (oral isotretinoin, 13-*cis*-retinoic acid) by inducing apoptosis in sebocytes[44,45] can also cause MGs to atrophy, since MGs are modified sebaceous glands.[46] Without functioning MGs, individuals taking these medications are prone to evaporative DE.[47-51]

1.1.2.4 Refractive Surgery

Patients who elect to undergo refractive surgery are also at risk for developing DE.[52,53] There is a measurable loss of corneal sensitivity[54,55] from iatrogenic nerve damage, causing a reduction in afferent input (sensory) and dampening of the efferent output (tear secretion).[53] There is also a loss of conjunctival goblet cell

density from the interaction of the suction ring on the eye.[56,57] Post-operative inflammation from an increase in metalloproteinase-9(MMP-9)[58] and inflammatory cytokine levels[59] in the tears can trigger symptoms and exacerbate pre-existing DE problems.[60] The incidence of developing chronic DE from refractive surgery is generally low (5.0% for PRK, 0.8% for LASIK), with many patients recovering tear function and symptoms 12 months after surgery.[61] However, individuals presenting with impaired tear film and chronic DE prior to surgery generally need a longer recovery time.[61] Post-operative DE is not isolated to refractive surgery, as it is also a very common side effect following cataract surgery.[62,63]

1.1.2.5 Environment Stressors

The environment can play a significant role in precipitating and exacerbating DE disease.[1] An increase in inflammatory cytokines, osmolarity, corneal staining, and MMP-9 can be observed after spending 2 hours in a dry environment.[64] An in-flight airplane cabin with low humidity can significantly decrease tear stability and tear volume, as well as increase corneal staining.[65] In mice, MG basal acinar cell proliferation can be triggered by placing the animals in a dry environment. This indicates that the environment can have a direct effect on the MGs.[66] Long hours of reading (either paper or electronic)[67] in a dry environment can increase the risk of DE in office employees, especially if they are contact lens (CL) wearers.[68] Dry eye test values can also be highly dependent on climate, with tear breakup time, corneal and conjunctival staining varying significantly between Atlantic and Continental regions.[69]

1.1.2.6 Contact Lens Wear

Inserting a foreign object into the tear film, such as a CL for the correction of refractive error or facilitating corneal wound healing, disrupts the layers of the tear film and alters the interaction of the tear film with the cornea.[70] Both conventional and silicone hydrogel CL materials can deposit with tear film components[71] and cause denaturation[72] of tear lysozyme and degradation of tear film lipids.[73] Deposition of protein and lipids can illicit an immune response in the form of giant papillary conjunctivitis.[74] Discomfort and dryness

are primary reasons why patients discontinue CL wear,[75] and DE plays a large role in contributing to the complex nature of CL discomfort.[76]

1.1.2.7 Topical Ophthalmic Medications

Antimicrobial agents are present in topical ophthalmic medication eye drops to preserve and extend their shelf-life.[77,78] The most common preservative is benzalkonium chloride (BAK).[77] It is a quaternary ammonium that is cheap to produce and is very effective at reducing microbial loads.[79] Unfortunately, BAK exacerbates ocular surface disease and DE in a number of ways; it promotes apoptosis in corneal epithelial cells,[77,80] breaks down tight junctions between epithelial cells,[80] reduces surface goblet cell numbers,[81] destabilizes the tear film,[82] and increases inflammatory cytokines.[83] Consequently, patients who use BAK-preserved topical ophthalmic medications for extended periods of time (e.g. for intraocular pressure management in glaucoma) will often need help with DE and ocular surface disease as well.[84] Newer formulations of topical medications with less toxic preservative systems may be more appropriate for long-term use.[85,86]

1.2 Etiology and Pathophysiology of Dry Eye

Dry eye is a complex and multifactorial disease. The 2007 DEWS I report defined DE to be

> "… a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface."[87]

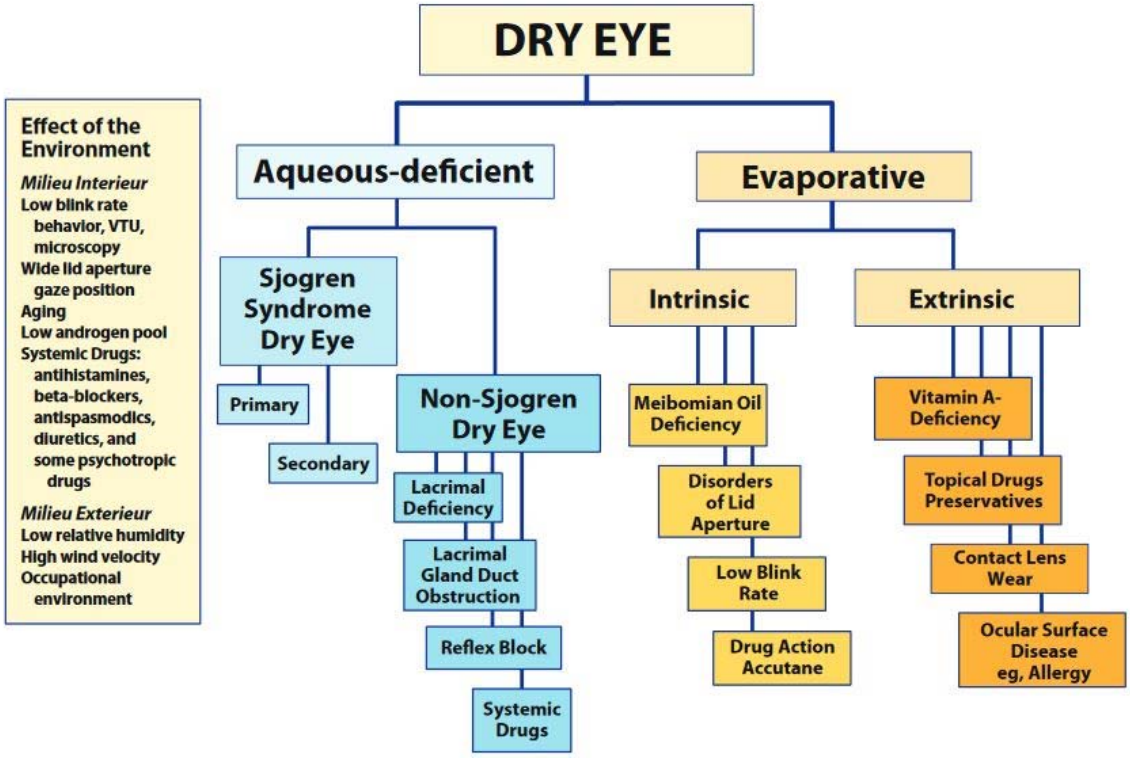


Figure 1-1: The major etiological categories of DE. Dry eye can be broadly categorized into two major etiologies. Within each etiology are sub categories that have potential to cause and exacerbate DE. This figure highlights the complex multifactorial nature of DE etiology. Image from Lemp MA, Baudouin C, Baum J, Dogru M, Foulks G, Kinoshita S, Laibson P, McCulley JP, Murube J, Pflugfelder SC, Rolando M, Toda I. 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;5:75-92.

The etiology of DE can be classified into two broad categories: aqueous deficient and evaporative DE (Figure 1-1).[87] The aqueous deficient category can be further divided into SS-DE, and Non-SS DE. Sjögren's syndrome DE can be further categorized into either primary or secondary. Non-SS DE etiology can be further broken down into lacrimal deficiency, lacrimal gland duct obstruction, reflex block, and systemic drugs.[87]

The evaporative DE category consists of intrinsic and extrinsic factors. Intrinsic factors that can cause evaporative DE include MG oil deficiency, lid aperture disorders, low blink rate, and drug action of isotretinoin.[87] Extrinsic factors include vitamin A deficiency, topical drug preservatives, CL wear, and ocular surface disease.[87]

Aqueous deficient and evaporative DE are not necessarily mutually exclusive. A patient could have a combination of intrinsic and extrinsic evaporative factors (e.g. lax lids and be using BAK-preserved medications), along with several aqueous deficient factors (e.g. primary SS, and on antidepressants). This can potentially complicate the diagnosis and management of DE disease.

1.2.1 Aqueous Deficiency

1.2.1.1 Sjögren's Syndrome

Infiltration of lymphocytes into the exocrine glands is a characteristic of SS,[88] and SS is a well-established cause for aqueous deficient DE.[37,89] Some clinical manifestations of SS include severe DE,[90] dry mouth,[91] and dry skin.[92] In addition to dryness, individuals with SS often exhibit physical fatigue and mental depression as well.[93] Like other rheumatic diseases, SS cannot be characterized by a single defining trait. The diagnosis is made after an assessment of multiple systems,[94] in particular the ocular surface, salivary glands, and serum antibodies.[95]

The classification of SS first started in 1986,[95] and has been revised several times since then. The European community had first established a consensus-based criteria in 1993,[96] which was subsequently validated in 1996.[97] A revision was conducted in a joint-effort with an American group of experts to improve on classifying primary and secondary SS, resulting in the American-European Consensus Group (AECG) classification criteria.[94] One of the criticisms of the AECG classification criteria was that it included subjective patient responses as a criterion, and so the classification criteria were revised once again by the American College of Rheumatology (ACR) in 2012.[98] The agreement between the AECG and the ACR

classification sets is mixed, with one study reporting fairly good concordance,[99] but two reporting only moderate agreement.[100,101]

The AECG and the ACR classification criteria are as follows:

The AECG classification criteria:[94]

I. Ocular symptoms: a positive response to at least one of the following questions:

1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than 3 times a day?

II. Oral symptoms: a positive response to at least one of the following questions:

1. Have you had a daily feeling of dry mouth for more than 3 months?
2. Have you had recurrently or persistently swollen salivary glands as an adult?
3. Do you frequently drink liquids to aid in swallowing dry food?

III. Ocular signs – that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests:

1. Schirmer's I test, performed without anesthesia (≤5mm in 5 minutes)
2. Rose Bengal score or other ocular dye score (≥4 according to van Bijsterveld's scoring system)

IV. Histopathology: In minor salivary glands (obtained through normal appearing mucosa) focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score ≥1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm^2 of glandular tissue.

V. Salivary gland involvement: objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests:

1. Unstimulated whole salivary flow (≤1.5ml in 15 minutes)
2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts
3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer

VI. Antibodies: presence in the serum of the following antibodies:

1. Antibodies to Ro(SSA) or La(SSB) antigens, or both

The ACR classification criteria:[98]

1. Positive serum anti-SSA and/or anti-SSB or (positive rheumatoid factor and ANA ≥1:320)
2. Ocular staining score ≥3 (using the Sjögren's International Collaborative Clinical Alliance grading scale)
3. Presence of focal lymphocytic sialadenitis with focus score ≥1 focus/4mm^2 in labial salivary gland biopsies.

In either case, the management of SS-DE often involve a combination of topical and oral anti-inflammatory agents, immunomodulatory agents, punctal plugs, and topical autologous serum.[102]

1.2.1.2 Lacrimal Gland Deficiency

The lacrimal gland is characterized by tubuloacinar, exocrine cells.[103] The entire gland is an almond-shaped structure. Within the gland, acini units comprised of pyramid-shaped cells secrete water, electrolytes, proteins, and mucins from the apical membrane into the lumen of an excretory duct, and into the tear film.[103] The basolateral membrane of the acinar cells contain receptors for peptides, hormones, and neurotransmitters that regulate the secretory processes of the gland.[103]

The sensory nerves innervating the ocular surface originates from the ophthalmic branch of the trigeminal nerve,[104] and the excitation of these nerves results in stimulation of lacrimation.[105,106] The cornea is one of the most densely innervated surfaces of the human body,[107] comprising of mechanoreceptors, mechano-nociceptors, polymodal nociceptors, and cold receptors.[104] Afferent signals from the ocular surface are processed in the superior salivary nucleus (lacrimal nucleus) of the facial nerve.[108] The superior salivary nucleus then supply efferent signals to the lacrimal gland.[108] Eliminating sensory input at the trigeminal nerve[109] and disrupting the efferent pathway [110] reduces lacrimation. This is often the case in refractive surgery, where the corneal nerves are often damaged, leading to the loss of corneal sensitivity,[54,55] and negatively affecting lacrimation.[53]

Aging has an impact on the neural regulation of the lacrimal gland. Corneal sensitivity decreases sharply after the age of 50,[111] and the reduction in sensory input from the ocular surface may be responsible for the decreased output of the lacrimal gland. Structural changes to the lacrimal gland,[112] decrease in overall innervation[113,114] and infiltration of mast cells and lymphocytes[113,114] are a result of aging in the lacrimal gland.[115]

Lacrimal deficiency can be due to congenital alacrima, a condition that is characterized by a complete absence or underdeveloped lacrimal gland.[116] In one case, a 5 year old girl had presented with a lack of tearing when crying. Her tear stability was less than 2 seconds, and had corneal epithelial erosions and conjunctival epitheliopathy. Magnetic resonance imaging had revealed a complete absence of a lacrimal

gland in the left eye, and a severely underdeveloped gland in the right eye.[116] She was managed with artificial tears and cyclosporine 0.05%.[116] Alacrima is also a clinical feature of Allgrove syndrome, an autosomal recessive congenital disease additionally characterized by Addison's disease and achalasia.[117,118]

1.2.1.3 Lacrimal Excretory Duct Obstruction

Since the lacrimal gland excretes into the tear film through a lacrimal excretory duct, any obstruction of the ducts could result in a decreased supply of tear fluid to the ocular surface. The cauterization of the lacrimal excretory duct in rabbits increased tear film osmolarity, decreased goblet cell density and decreased corneal epithelial glycogen levels.[119] However, this could not be completely attributed to the cautery, since the harderian gland and nictitating membranes were also simultaneous excised in this experiment.[119]

1.2.2 Evaporative Dry Eye

The second major etiological category relates to evaporative disorders of the tear film. Intrinsic factors that can cause evaporative DE include MG oil deficiency, lid aperture disorders, low blink rate, and the reaction to taking isotretinoin and its derivatives. Extrinsic factors include vitamin A deficiency, topical drug preservatives, CL wear, and ocular surface disease (Figure 1-1).

1.2.2.1 Lid Aperture Disorders

At this time, there are little to no studies that adequately examine the relationship between aperture disorders and DE. Without adequate closure of the eyelids, the ocular surface is exposed to desiccation. Incomplete blinking, lagophthalmos, nocturnal lagophthalmos, exophthalmos, ectropion, entropion and facial palsies are all sources of incomplete eyelid closure that may precipitate DE symptoms and signs. Additionally, without the proper apposition of the eyelids against the eye, the MG orifices are disconnected from the tear film, along with a loss of physical support for the inferior tear meniscus.

Idiopathic facial palsy (Bell's palsy) may result in the inability of the patient to close their eye completely on the affected side.[120] The management of Bell's palsy often involves eye patching and constant lubrication

to avoid corneal complications.[121,122] Other conditions such as lagophthalmos, ectropion, may occasionally require additional therapies such as eyelid weight loading, tarsorraphy, taping and lateral canthoplasty to restore eyelid structure and function. Patients with more lower lid laxity (but without ectropion, entropion, lagophthalmos) tend to be symptomatic for DE.[123]

1.2.2.2 Low Blink Rates

Blinking refreshes and redistributes the tear film over the ocular surface.[124] Patients with DE generally blink more frequently than people without DE.[125,126] This is due to the tear film destabilizing faster in DE patients,[127] which frequently stimulates the blinking reflex. Using a computer reduces the blink rate for both DE and normal individuals.[128] Also, a separate study found a higher frequency of incomplete blinks when using computers as opposed to printed hard copy paper.[129] With a reduction in blink rate, the tear film is not replenished as frequently and the ocular surface is constantly exposed to desiccating stress.

1.2.2.3 Meibomian Gland Disease

1.2.2.3.1 Congenital Absence

Meibomian gland disease contributes to MG oil deficiency, resulting in evaporative DE.[130] Meibomian gland disease can be the result of a lack of MGs from a rare genetic disorder called ectrodactyly ectodermal dysplasia cleft lip/palate (EEC syndrome).[131] A case report documented a case of EEC syndrome in a 22 year old Japanese male, in which there were no MG orifices observed at the slit lamp, and an absence of MGs upon transillumination.[131] This patient had previously managed his DE by using unpreserved artificial tears 6x/day and 0.1% vitamin A drops 4x/day, but reported symptoms worsening when viewing a computer screening or being in dry, windy, dirty environments.[131]

1.2.2.3.2 Meibomian Gland Neoplasia

Meibomian gland neoplasia is another rare aspect of MG disease. Sebaceous gland carcinomas are a group of cancers that can arise from the MGs, glands of Zeis or glands associated with the caruncle.[132] However,

tumours of the MGs must not be confused with chalazia – a common localized, lipogranulomatous lesion within the MGs.[133]

1.3 Meibomian Gland Dysfunction

1.3.1 Etiology and Pathophysiology of Meibomian Gland Dysfunction

The most common contributor to MG disease is MG dysfunction (MGD). The 2011 International Workshop on Meibomian Gland Dysfunctional categorized MGD into low (hyposecretory) or high delivery (hypersecretory) states.[130] Terms such as meibomitis and meibomianitis are no longer used since MGD can occur in the absence of inflammation.[130,134] Figure 1-2 displays the relationship between MG disease and MGD, as well as some of their etiologies.

Hypersecretory MGD is characterized by an excessive secretion of lipids, and appears to be related to an excess of androgens,[135] testosterone in particular, as it promotes sebaceous cell proliferation and differentiation.[136] In contrast, abnormally low delivery of meibum can be secondary to certain types of medications that cause MG atrophy.[130] As previously discussed, oral isotretinoin for the treatment of acne vulgaris induces apoptosis in sebocytes, resulting in a decrease in sebum production.[44,45] Since MGs are modified sebaceous glands,[46] the use of isotretinoin causes MGs to atrophy and reduce meibum output.[47-51] Decreased delivery of meibum can be due to a state of androgen deficiency, which can result from anti-androgen therapy (as for prostate cancer therapy[137] or acne treatment[138]). Without sufficient androgen levels, MG activity is supressed and meibum production is decreased.[33]

Low delivery of meibum may also be due to MG obstruction.[130] Obstructive MGD is probably the most common type of MGD.[130] Obstructive MGD can be cicatricial or non-cicatricial in nature. Cicatricial obstructive MGD can be secondary to trachoma, ocular pemphigoid, erythema multiforme, and atopy, and non-cicatricial MGD include seborrheic dermatitis, rosacea, atopy and psoriasis.[130]

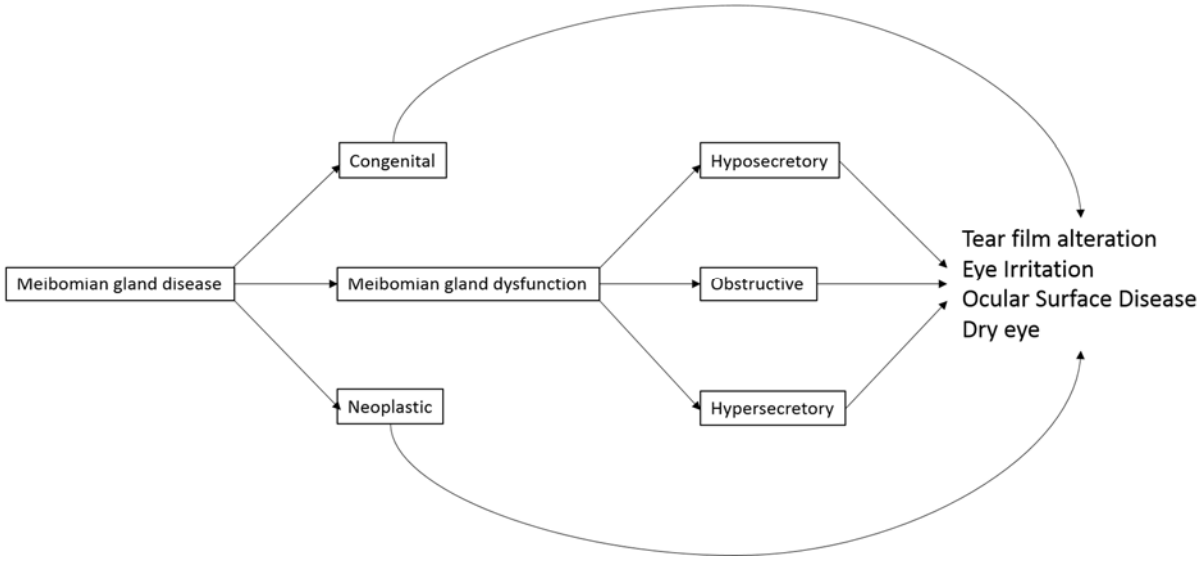


Figure 1-2: The classification of MG disease and MGD. Meibomian gland disease is an umbrella term that encompasses MGD. There are three subtypes of MGD; hyposecretory and obstructive both result in low-delivery of meibum, hypersecretory refers to high-delivery of meibum. All of these can result in alterations to the tear film, causing DE and ocular surface disease. The figure is adapted from Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1930-1937.

The most recent definition of MGD by the 2011 International Workshop on Meibomian Gland Dysfunction is as follows:[130]

> "Meibomian gland dysfunction is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. This may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease."

1.3.1.1 Anatomy of Meibomian Glands

Meibomian glands are long, modified sebaceous glands imbedded within the tarsal plate.[46] There are approximately 25 to 40 (median 31) in the superior eyelid, and 20 to 30 (median 26) in the inferior eyelid.[46] The array of glands are arranged vertically, with the closed end positioned most distal to the eyelid margin. The opening of the glands are located posterior to Marx's line. Each single gland consists of a central duct with numerous small acini clusters connected to it via small ductules (Figure 1-3). The term "chain of onions" has been used to describe the layout of a single gland.[46]

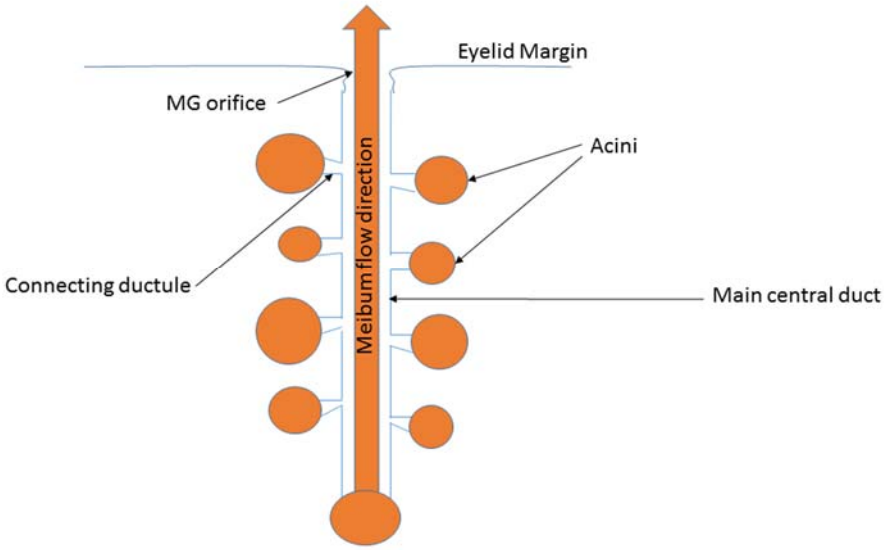


Figure 1-3: A MG functional unit consists of a central duct, with multiple acini units connected to it. Meibum is produced at the acini units, which travels to the central duct, and eventually out the MG orifice at the eyelid margin.

The meibum production begins at the acinus units. The acinus are approximately 150-200μm in diameter and consist of secretory cells termed meibocytes. The maturation of meibocytes begin at the basement membrane at the periphery of the acinus.[46] The basal cells serve as progenitor and proliferates to constantly produce new meibocytes. As meibocytes mature they migrate towards the center of the acinus.[46] The end of maturation is marked with the disintegration of the cell membranes, as they release their contents into

the lumen of the ductal system (holocrine secretion).[46] The constant replacement of meibocytes ensures this process is continuous. In rats, the turnover for meibocytes is approximately 4 days,[139] this may be slightly different in humans. The regulation of meibocyte proliferation and differentiation appears to be driven in-part by CD147, a transmembrane protein that also serves to regulate MMP-9s.[140]

1.3.1.2 Hormonal Regulation

The production of meibum is regulated by numerous mechanisms. Regulation by androgens and estrogens have been studied extensively.[141] Some evidence suggests that the MGs are innervated by both the sympathetic[142] and parasympathetic[143] autonomic nervous systems. In mice with ovaries removed, there was an increase in expression of neuropeptide Y (associated with the sympathetic system) and decrease in vasoactive intestinal polypeptide (associated with the parasympathetic system) in the nerve fibers of the MGs.[144] This shows that hormones can have an influence on autonomic nervous control of MGs, complicating the understanding of its regulation.[144]

Secreted meibum travels through the connecting ductules, which are approximately 150μm in length and 30-50μm in diameter. The connecting ductules transition into the central duct, which is 100-150μm in diameter, with the length traversing the length of the eyelid.[46] These ducts are characterized by a four-layered stratified squamous epithelium, containing keratohyalin granules. The excretory duct of the MG is the final portion of the gland that meibum travels through before it reaches the opening at the eyelid margin.[46] The excretory duct differs from the rest of the duct system in that it contains an ingrowth of keratinized epithelium from the eyelid margin. This is a key anatomical feature that is responsible for the obstruction of the MG orifice when keratinization is upregulated (hyperkeratinization).

1.3.1.3 Hyperkeratinization

The results of a study by Jester showed that MGs from both humans and rabbits were capable of expressing keratin proteins, indicating that keratinization is a process inherent to MGs.[145] Hyperkeratinization of the eyelid margin and MG orifice epithelium can cause orifice narrowing and create a stenosis that results in

decreased delivery of meibum to the tear film. There are several triggers for hyperkeratinization.[46] Hyperkeratinization can be due to anti-androgen therapies,[146] CL wear,[147] or artificially induced by topical epinephrine.[148] Furthermore, hyperkeratinization of the eyelid margin epithelium was thought to contribute to narrowing and pouting of the MG orifices.[149] Obstruction of the orifices have been shown to induce atrophic changes in MGs in mice[150] and rabbit models.[148,151] While hyperkeratinization is a major contributor to obstructive MGD, not all MGD arise from obstruction. In a study that compared 2 year old versus 5 month old mice,[152] Parfitt et al. had found that the lack of meibum production was due to a loss of acinar progenitor cells. The keratin markers showed no hyperkeratinization of the excretory duct of the MGs.[152]

1.3.1.4 Meibum Quality Changes

Change in meibum quality is another characteristic of MGD. The physical quality of meibum can be assessed clinically by applying pressure to the eyelid margins (see Figure 1-4). Normal meibum is an optically clear and oily fluid, resembling the consistency of cooking oil. As MGD severity progresses, the appearance of meibum can take on a thicker, opaque, creamy consistency that is relatively thicker in viscosity, resembling toothpaste.

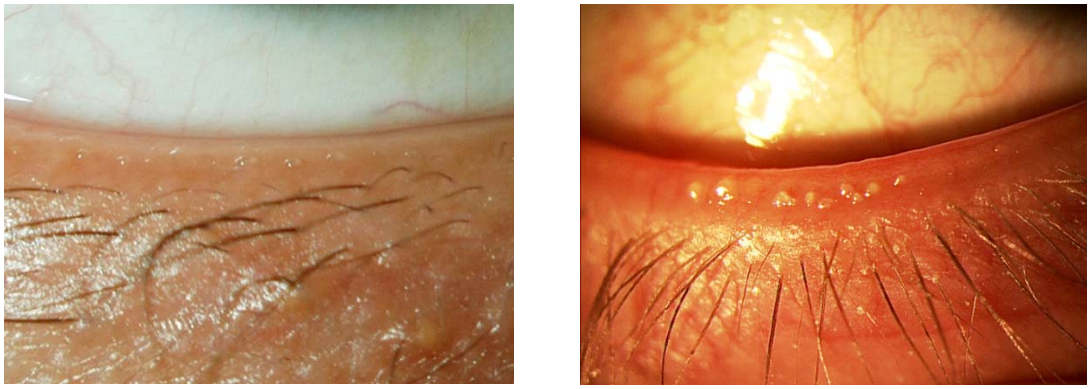


Figure 1-4: Left, upon expression, healthy meibum should appear clear and liquid in consistency. Right, altered meibum quality appears white, paste-like and turbid.

The collection of meibum is the first step to analyzing meibum lipid composition. Meibum can be collected from humans in a number of ways.[153]

1) Spatula: pressure is applied to the eyelid margin and a sterile spatula is used to collect the meibum. A disadvantage to this procedure is that other extraneous cells and debris may also be collected as well.
2) Microcapillary pipettes: pressure is applied to the eyelid margin to express a small "pool" of meibum. A microcapillary tube is positioned on top of the meibum and drawn into the tube. While this is one purest ways meibum can be obtained, a disadvantage to this procedure is that very thick meibum may be very difficult to collect and then also to remove from the tube.
3) Paper strips and swabs: pressure is applied to the eyelid margins to express oils and an absorbent strip of paper is applied to the eyelid margins to collect it. Lipids can then be extracted from the paper. A disadvantage to this technique, due to the large surface area, is that it may also incidentally collect lipids from other ocular surface cells.
4) Chalazion curette: a chalazion curette is able to collect meibum in a manner similar to the spatula described above.

The methods for analyzing meibum are various as well. In general, chromatography (paper, thin layer, gas, liquid), mass spectroscopy, spectroscopy, nuclear magnetic resonance, raman spectroscopy, infrared spectroscopy, and chemical photometric assays are some methods in which the composition of meibum can be derived.[153]

It is generally observed that younger individuals have lower meibum viscosity.[154] Borchman et al. studied the relationship between the physical property of meibum and age using infrared spectroscopy[155] and found a significant decrease in meibum hydrocarbon chain order, from 48% *trans* rotamers at birth to approximately 30% *trans* rotamers at approximately 85 years old.

The difference in meibum composition may explain the difference in tear film stability observed between infants and adults.[156] In a separate study, Borchman et al. identified several areas by H-NMR that appear to change with age. The amount of -CH_3, C=C, and degree of lipid oxidation increased over time. The increase in C=C bonds indicated a higher degree of branching in lipid structure. As a result, lipid molecules do not stack as close and van der Waal's forces become diminished, making it easy for lipid-lipid interactions to be broken. This was the hypothesized reason for decreased tear stability in adults.[156]

Borchman et al. also compared the difference in meibum quality between normal individuals and those with MGD.[157] The study found that meibum from MGD participants had a higher lipid order than normal meibum, which resulted in higher phase-transition temperatures. Lipids that are more tightly packed together interact with greater van der Waal's forces, resulting in higher melting temperature. This is clinically relevant as treatment for MGD patients typically involve the application of heat to the eyelid margins to melt the meibum.[158] An analogy used in this study is that the hydrocarbon stiffness of meibum in MGD is somewhere halfway between olive oil and butter.

A more recent study[159] found that meibum from participants with MGD had more insoluble inclusion bodies than normals. These inclusion bodies were stainable with Amido Black, suggesting that they were composed of proteins. Furthermore, these inclusion bodies also stained with PanCK and CK10 antibodies, indicating that they were cytokeratins. Meibum from MGD were also found to be much less mechanically resistant than normals. This was shown by mechanically stressing (applying pressure using a coverslip against a glass slide) the meibum. Meibum from normals was able to retain its integrity under pressure, but meibum from MGD participants disintegrated.[159]

Meibum forms the majority of the tear film lipid layer and is thought to prevent evaporation of the tear film.[160] However, recent evidence suggests that the main function of the lipid layer is to maintain a thin tear film while preventing it from collapsing.[161]

1.3.1.5 Non-Obvious Meibomian Gland Dysfunction

As previously discussed, MGD can occur in the absence of inflammation.[134] The term non-obvious MGD (NOMGD) is used to describe a form of obstructive MGD that is relatively inconspicuous, and is characterized by the absence of lid margin notching and inflammation.[134] However, after applying pressure to the eyelid margins, the orifices may yield solidified meibum plugs or nothing at all. To ensure that NOMGD is not missed, diagnostic expression of the MGs is encouraged as part of a regular ophthalmic examination.[134] Diagnosing NOMGD and managing it early may prevent it from progressing to obvious MGD.[134]

1.3.1.6 Isotretinoin Use

The use of isotreinoin and their impact on MGs have been discussed in the section on Systemic Conditions and Medications.

1.3.2 Prevalence and Risk Factors for Meibomian Gland Dysfunction

The prevalence of MGD from population studies ranges from 3.5% up to 69.3%.[162] The large variation may be due to varying definitions of MGD and ethnic groups. Higher prevalence of MGD appears to occur in Asian populations.[162] The prevalence of evaporative dry eye caused by MGD is higher than dry eye resulting from aqueous deficiency.[163] The ophthalmic risk factors for MGD previously identified were aniridia, chronic blepharitis, CL wear, *Demodex* blepharitis, eyelid tattooing, floppy eyelid syndrome, giant papillary conjunctivitis, ichthyosis, Salzmann's nodular corneal degeneration, and trachoma.[162]

1.3.2.1 Congenital Aniridia

Congenital aniridia is a condition caused by insufficiency of PAX6, a gene responsible for the development of various structures of the eye, including the MGs.[164,165] As a result, aniridia is often accompanied by problems ranging from cataracts, glaucoma, foveal hypoplasia, optic nerve hypoplasia, and ocular surface

disease. Ocular surface disease occurs in the form of vascularization and keratinization of the corneal due to limbal stem cell deficiency.[164]

1.3.2.2 Ichthyosis Follicularis

Ichthyosis follicularis is a condition that is often accompanied by alopecia and photophobia (IFAP).[166] This is a rare X-linked disease that is associated with MGD.[162] One recent case report describes MGD in a father and daughter, both with IFAP syndrome.[167]

1.3.2.3 Eyelid Tattooing

Eyelid tattooing has a possible association with MGD. This finding is based on a case report published in 2005 by Kojima et al., describing complete loss of MGs in a 45 year old female who had undergone eyelid tattooing.[168] A more recent study by Lee et al.[169] confirms this association, by showing that tear breakup time, fluorescein staining, and MG atrophy was worse in the tattoo group than the control group. The authors propose three reasons why tattooing may cause MG atrophy; the needle used to inject tattoo ink into the dermis may cause mechanical trauma to the eyelid margins, the ink may contribute to substance toxicity, and thirdly, tattoo ink may obstruct the ducts and the gland openings.

1.3.2.4 Floppy Eyelid Syndrome

Floppy eyelid syndrome is possibly related to MGD, based on a case report published in 1987 by Gonnering et al.[170] A PubMed search for "meibomian" and "floppy eyelid syndrome" revealed another paper published in 1994 by Netland et al.,[171] that found MG abnormalities while characterizing tarsal elastin in floppy eyelid syndrome.

1.3.2.5 Contact Lens Wear

Whether or not CL wear causes MGD is still unclear, with many studies yielding conflicting and mixed results. Ong et al.[172] examined the proportion of individuals with MGD in CL wearers versus non-CL wearers and found no significant difference between the two groups. Arita et al.[173] compared MG atrophy

in CL wearers and non-CL wearers and found that CL wearers had a significantly higher amount of atrophy than non-CL wearers. Furthermore, they also found that duration of CL wear and MG atrophy were significantly correlated. Marren et al.[174] found no correlation between CL wear and MGD. Arita et al.[175] found that CL-induced allergic conjunctivitis caused MG distortion similar to those found in perennial allergic conjunctivitis. Machalinksa et al.[176] found a significant correlation between meibum quality, expressibility and duration of soft CL wear in an age-matched study. A case-control sex-matched study by Pucker et al.[177] found an inconclusive association between CL wear and MG atrophy. As it stands currently, the association of CL wear to MGD remains equivocal.

1.4 Clinical Tests for Dry Eye

The diagnosis and management of DE frequently involves the assessment of various aspects; patient symptoms, the quality of the tear film, and the integrity of the ocular surface. Symptom assessment is important, since DE disease is primarily symptom driven. Assessment of the tears and ocular surface is important, since chronic desiccation of the ocular surface may result in corneal scarring and loss of visual function. However, symptom measurements often do not correlate with objective DE measurements.[178-180] This suggests that symptoms alone are not enough to diagnose and manage DE, and that DE assessment must include objective DE measurements.

1.4.1 Subjective Assessments

Symptom assessment is usually conducted subjectively using questionnaires that may assess purely DE symptoms (examples include the Standard Patient Evaluation of Eye Dryness (SPEED) questionnaire [181] and Symptom Assessment in Dry Eye (SANDE) questionnaire[182]), a combination of DE symptoms and quality of life (Ocular Surface Disease Index, (OSDI)[183]) or questionnaires that focus mostly on quality of life (Impact of Dry Eye on Everyday Life (IDEEL)[184]). The advantage of using questionnaires is that they are non-invasive, and there are no other instruments that can provide a researcher or clinician information about a patient's morbidity or quality of life.[185]

The main problem with using questionnaires is that because they provide a subjective outcome, there is no external objective reference.[185] Patients' scores cannot be compared to another, since patients may have different sensitivities and perceptions of pain and discomfort.[185] Another is that patients are prone to psychological bias. Their responses on the questionnaire can be influenced by their mood. Language barriers can also be a problem, if a patient is confused or does not understand the questions.

Questionnaires measure an underlying latent construct. A latent construct is an unobservable trait that is the subject of measurement. In this case, the latent construct is DE disease. Latent constructs can be quantified by querying the different aspects of the construct, and then analyzing the questions and responses with Rasch analysis[186] or Item Response Theory to determine how well the items fit the construct. Questionnaires must be tested to show that they can accurately and reliably measure the underlying latent construct in a unidimensional manner.[186] For example, the scale of the instrument should scale linearly with the disease so that a high attribute of the latent construct should be reflected as a high measurement on the scale, and a low attribute of the latent construct should be reflected as a low measurement on the scale. Incremental increase in the latent construct should be reflected with a proportional increment in the scale. In this case, DE questionnaires attempt to query the severity of the disease by querying ocular symptoms or quality of life measures with dry eye.

1.4.1.1 Ocular Surface Disease Index (OSDI)

A validated DE questionnaire that is commonly used in clinical research and patient care is the Ocular Surface Disease Index (OSDI).[183,187] It contains items that queries a combination of DE symptoms and quality of life items in the previous week that are relevant to DE disease. The OSDI is one of the few questionnaires that meets the Food and Drug Administration (FDA) criteria for patient-reported outcomes (PRO) in quality of life measures.[188] A composite score is generated based on the responses, with a higher score indicating more severe disease. A potential drawback for the OSDI is that categories for each individual item may not scale linearly, which can affect the scaling properties of this instrument.[189] A search for published studies with the terms "Ocular Surface Disease Index" between Jan 01 2015 – Dec 31 2015

in PubMed revealed 125 studies. Some of the studies that used the OSDI included one that studied corneal and conjunctival sensitivity in rosacea patients (n=92),[190] ocular surface disease in diabetic peripheral neuropathy patients (n=34),[191] and the relationship between DE and depression (n=94).[192]

1.4.1.2 Symptom Assessment in Dry Eye (SANDE)

The SANDE questionnaire was developed by Schaumberg et al.[182] and is different than the OSDI in that it only queries the frequency and severity of symptoms on average using two visual analogue scales. The questionnaire also yields a composite score that indicates disease severity. A study by Amparo et al.[193] compared the SANDE to the OSDI and found a significant correlation ($R=0.64$, $p < 0.0001$) at baseline and follow up ($R = 0.47$, $p < 0.0001$). Bland-Altman plots shows bias of -1.5 units at baseline, and 1.8 units at followup. The authors conclude that the SANDE is a fast and simple questionnaire to conduct, with performance similar to the OSDI.

1.4.2 Objective Assessments

Following subjective assessments, objective clinical tests are conducted. Some of these tests include assessments of tear stability, tear film osmolarity, lipid layer thickness, MG function, ocular surface integrity (with vital dyes), tear volume, tear secretion, eyelid margins and the lid wiper region.[194]

1.4.2.1 Tear Film Osmolarity

Tear film osmolarity may play a vital role in the pathogenesis of DE disease.[87] The increase in concentration of solutes creates a hyperosmotic environment for the ocular surface. This increase in tear osmolarity has been found to increase the production of inflammatory cytokines in corneal epithelial cells in an *in-vitro* study conducted by Igarashi et al.[195] However, the osmolarity values used by Igarashi et al. to induce inflammation (400mOsm - 800mOsm) were beyond the range typically measured *in vivo*, even in SS,[196] where DE disease is severe.

There are three different methods for measuring tear osmolarity; freezing point depression,[197] vapour pressure,[198] and electrical impedance.[198] Freezing point depression osmolarity is based on the principle that the presence of solutes in a solution decreases its freezing point.[199] Vapour pressure osmometers is based on the principle that vapour pressure is lower in a solution that contains more solutes.[200] Electrical impedance osmometry measures the electrical conductivity of a solution, which changes based on the ionic concentration.[199]

Vapour pressure and freezing point depression osmometry can be challenging to conduct in a clinical setting. Vapour pressure osmometry requires a relatively large amount of tear fluid for measurement (approximately 0.8μL - 2μL),[201] and freezing point depression is expensive and requires specialized research equipment. An electrical impedance osmometer is commercially available (TearLab® Osmolarity System, TearLab® Corporation, San Diego, CA, USA) for clinical DE testing.[202] The TearLab® has been compared with freezing point depression[199] and vapour pressure[198] techniques and was found to exhibit good correlation with the two osmometers.

The TearLab® instrument was described to be the "single best test" for the diagnosis of DE.[203] Using a cutoff of 308 mOsms/L, sensitivity and specificity for mild DE disease was found to be 88% and 75% respectively.[203] A cutoff of 316 mOsms/L can be used for more moderate/severe patients, with sensitivity and specificity of 69% and 92% respectively.[204] However, the performance of this instrument for DE diagnosis remains mixed. Bunya et al.[205] found no significant difference in mean osmolarity between participants with SS, blepharitis, and control groups. Variability in osmolarity was increased in the SS and blepharitis group compared to the control.[205] Tear film osmolarity was found to correlate negatively with tear meniscus height,[206] but was not impacted by refractive surgery.[207] Another study found no correlation between tear osmolarity and symptoms,[208] and there was no detectable difference in tear osmolarity between women using CLs and oral contraceptive pills and those who were not.[209]

1.4.2.2 Tear Film Stability

Tear film stability represents the duration in which the tear film remains spread and fully covers the ocular surface. The tear film destabilizes when the most anterior lipid layer collapses onto the corneal surface. A summary of various techniques employed in the study of tear film breakup time has been previously reported.[210] Tear stability can be measured by instilling sodium fluorescein and observing the tear film down the slit lamp microscope with cobalt blue light and a Wratten 12 yellow barrier filter. The time for when a discontinuity is observed during the interblink interval is reported.[210] There are a number of methods in which fluorescein can be instilled. One method is to use a standard ophthalmic fluorescein strip, where the tip is wetted and instilled into the eye at the inferior fornix. A problem with this method is that due to an uncontrolled volume of fluorescein instilled, this method may artificially increase the tear volume on the ocular surface, affecting its repeatability.[211] To minimize this problem, there are thinner (1mm) fluorescein strips (Dry Eye Test, Amcom Laboratories, Saint Louis, MO, USA)[212] that limit the amount of fluorescein and volume that could be instilled into the eye. Using a pipette to instill a controlled amount of fluorescein is another way to improve repeatability of tear breakup time measurements.[213]

The addition of sodium fluorescein has also been shown to artificially destabilize the tear film.[214] There are methods that do not require the addition of fluorescein or extraneous fluids to assess tear film stability (so-called "non-invasive" methods).[215] The procedure generally involves the projection of a mire, grid or Placido disk rings onto the anterior tear film, and then observing for when distortions occur in the reflection.[216] The limitation of this procedure is that bright projections and sustained eye-opening may cause reflex tearing.[217] A number of other methods using interferometry, confocal microscopy, visual acuity, and aberrometry, have been used to study tear film stability.[210]

1.4.2.3 Tear Film Lipid Layer Thickness

The majority of the lipids in the tear film originate from the MGs.[218] The lipids form a thin layer on top of the tear film and act as a "blanket" to stabilize and reduce evaporation of the tear film.[219] The lipid layer

thickness (LLT) is approximately 100nm and can range from <60nm – 180nm.[220] Lipid layer thickness correlates significantly with fluorescein tear breakup time, Schirmer's test,[221] and with DE symptoms.[222] One commercially available device for quantifying tear film LLT is the LipiView (TearScience, Morrisville, North Carolina, USA). However, the LipiView LLT values showed no significant correlation with tear breakup time,[223] and was found to thin or not change after receiving a LipiFlow treatment (TearScience, Morrisville, North Carolina, USA).[224] The inter-observer coefficient of repeatability of the LipiView is 13nm, with an inter-observer coefficient of repeatability of 16nm.[225] In the same study, LLT did not correlate with corneal staining, tear breakup time, and DE symptoms.

1.4.2.4 Meibomian Gland Evaluation

The MGs are assessed by applying pressure to the eyelid margins and observing the quality of meibum that is expressed. Without applying pressure to express the glands, it can be difficult to assess the state of obstruction.[134] Typically, pressure to the eyelid margins is applied digitally, with the fingertip applied just below the base of the eyelash margin (Figure 1-5).

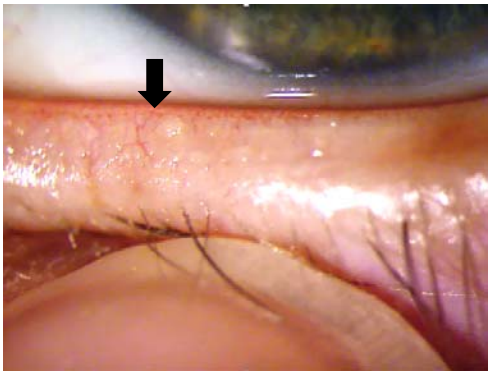


Figure 1-5: A common method to express meibum from MGs is to apply pressure using the leading edge of a finger against the base of the eyelashes. Note the paste-like meibum that is being expressed (black arrow).

A potential problem with this technique is that pressure exerted on the eyelids is uncontrolled and can vary between individuals. To remedy this, TearScience has developed a small hand held device with a spring

mechanism (Meibomian Gland Evaluator, MGE) that exerts a pressure of approximately 1.25g/mm^2 over an area of 40mm^2, to simulate the force of blinking on the MGs (Figure 1-6).

Figure 1-6: The MGE consists of a metallic body with a spring-mounted plastic tip. The leading edge of the plastic tip is depressed halfway against the inferior eyelid margin, for 10 seconds at the base of the eyelashes to express meibum from the MG orifices.

The leading white tip is approximately 15mm long and the total spring travel is 6mm. To ensure constant pressure, the white tip should travel approximately 3mm (or the halfway point) to maintain the pressure of 1.25g/mm^2 and not to exceed 6mm. The white tip should be held for approximately 10 seconds to ensure expressible meibum is expressed.

The MGE can be used to express 5 glands simultaneously and can be used to assess the temporal, central and or nasal portion of the eyelids (Figure 1-7).[226]

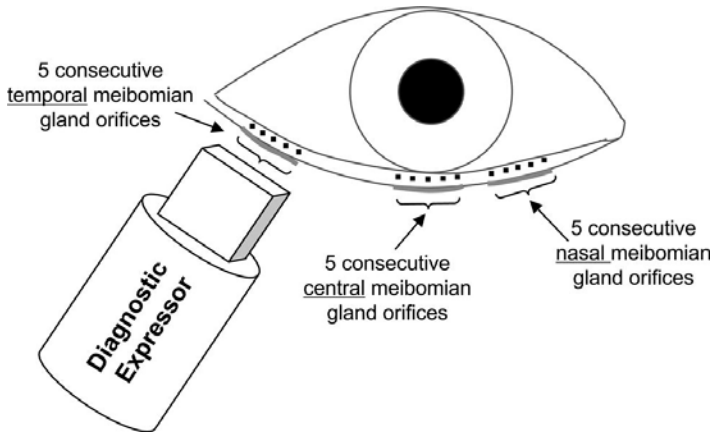


Figure 1-7: The MGE can be used to express five consecutive glands temporally, centrally, and nasally. Image from Blackie CA, Korb DR. The diurnal secretory characteristics of individual meibomian glands. *Cornea.* Jan 2010;29(1):34-38.

Each single gland can then be evaluated based on the type of meibum secretion that is observed. In the study by Friedland et al.,[227] secretions were graded from 0 to 3 (0: no secretion, 1: inspissated, 2: liquid coloured, 3: clear oil). The grades were then summed for a total out of 15 per each portion of eyelid, for a grand total out of 45 for the entire eyelid.[228] An additional parameter that can be obtained from this measurement is the "Meibomian Glands Yielding Liquid Secretions Score", where the number of glands with secretions of grade 2 or higher is counted.

Without an MGE, the MGs can still be assessed with digital pressure, but the pressure exerted will vary. A study[229] found that meibum expressed and graded using a cotton-tipped applicator was not significantly different than the MGE in MGD and non-MGD controls. Various grading scales have been used to grade the secretions.[230-232]

Manipulating the eyelids (e.g. for eyelid eversion) prior to assessing MG expression should be avoided, since applying pressure to the eyelids will manually express the contents of the MGs. For this same reason, the evaluation of meibum secretion on the superior lid on may be difficult since it typically requires some lid manipulation to observe the MG orifices.

1.4.2.5 Meibography and Meibomian Gland Imaging

(Section adapted from Ngo W, Srinivasan S, Jones L. Historical overview of imaging the meibomian glands. *Journal of Optometry* 2013;6:1-8.)[233]

Meibography relates to various methods of visualizing and imaging the MGs, and has become an important tool to monitor disease progress. There are a number of methods in which meibography can be conducted. The technique has evolved over the past few decades in parallel with advances in medical technology. Traditionally, the eyelids were transilluminated with a light source, and the transmitted infrared (IR) rays were captured with an IR camera.[234-236]

1.4.2.5.1 Lid transillumination

The technique of transilluminating the lid and observing it under the microscope was first described by Tapie in 1977,[234] by using an illumination probe typically used for intraocular vitreous surgery.[230,234,235] The tip of the probe was inserted behind the everted eyelid and the silhouette of the MGs was then observed on the other side. At the time, this was the only way of obtaining information about the morphology and physical characteristics of the MGs. Some major disadvantages of this technique was that the probe tip was small and sharp, causing uncomfortable heat, discomfort and pain to patients.[237] The transillumination area was also small, making it difficult to capture images of the entire length of the lid. Meibomian gland dropout in the lid were indicated by decreased transmission of light.

Fiber optic cables and other devices can be used as light sources for transillumination.[230,238-242] As the eyelid is everted over the fiber optic cable, it is transilluminated by the light that is conducted through the cable. Since fiber optic cables are smaller and thinner, it is more patient-friendly than solid, hand-held probes. Once the eyelid is transilluminated, the MGs and acini are revealed. The practitioner can then evaluate and record the appearance of the glands using some form of photography. Meiboscopy is the viewing of the MGs (using a tool like a Finoff transilluminator) without the use of photography, whereas meibography implies visualization and use of photography (film or digital). Photography of MGs can be conducted by

combining a photo slit lamp with high speed IR film.[236] The process usually requires IR film and the process of developing IR film can be very time consuming.

From the images, the number of MGs can be manually counted and the degree of MG dropout, or area of MG loss, can be measured. Lid transillumination has been used to study MG dropout in isotretinoin use for acne vulgaris,[230] blepharitis,[239] chronic blepharitis,[230] aging,[242] ocular surface disease and MGD,[232] and SS.[238] The practice of lid transillumination remains a key technique in studying MGs, but the use of IR film has been overshadowed by more advanced digital video systems. [243]

1.4.2.5.2 Video Meibography Systems

Video-meibography systems have addressed some of the disadvantages with IR photography. In 1994, Mathers et al. had developed a video-meibography system which allowed the transilluminated eyelid to be viewed in real-time on a computer.[243] A VHS recorder was used to record videos and individual frames from the video sequence was extracted for analysis.[243] The quality of the images was comparable to the images captured using IR camera, but without the inconvenience of developing IR film. However, the small and localized nature of the light source meant that it still required approximately 5 images to compose the entire eyelid.[243] This was remedied by fitting custom adaptors onto the transilluminator that allowed a wider area of light distribution. Yokoi et al. had designed an oblique T-shaped adaptor with an array of windows along the head of the adaptor that simultaneously facilitate eyelid eversion while transilluminating it underneath. This reduced the number of images to cover the entire eyelid from 5 to 3.[237] Recently, improvements in imaging technology have made it possible to conduct meibography without a transilluminating device.

1.4.2.5.3 Non-contact Meibography

Imaging the eyelid with an IR source and then capturing the image using an IR charge-coupled device (CCD) is the main principle behind non-contact meibography. Images of the entire eyelid can be obtained within a minute. A major advantage with non-contact meibography compared to previous methods is that

a transilluminating light source is no longer required, improving patient comfort significantly. Non-contact meibography is flexible and can be adapted from a slit lamp,[244] a security camera (Figure 1-8),[245] or even built into multi-purpose devices such as the Keratograph 4 (Figure 1-9)[246,247] and 5M (Figure 1-10) (OCULUS, Wetzlar, Germany).[248]

Figure 1-8: Left, an infrared security camera modified for meibography. A ring of infrared LEDs illuminate the eye, and the central camera unit captures infrared video to be displayed on a computer screen (not shown). Right, this image was captured by the modified security camera. The ring configuration of the infrared LEDs of the camera can be seen in the reflection of the cornea. Courtesy of Dr. Heiko Pult

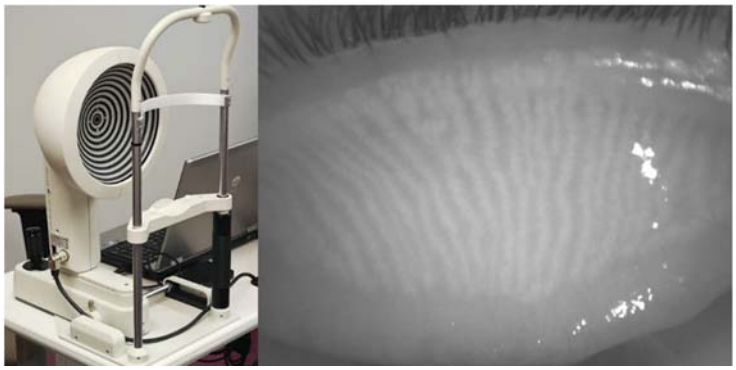


Figure 1-9: The OCULUS Keratograph 4 functions primarily as a corneal topographer, however the infrared diode intended for pupillometry can be used as an infrared illumination source for meibography (left). An everted superior eyelid reveals long thin MGs running vertically on the palpebral side as visualized by the OCULUS Keratograph 4 in infrared light (right).

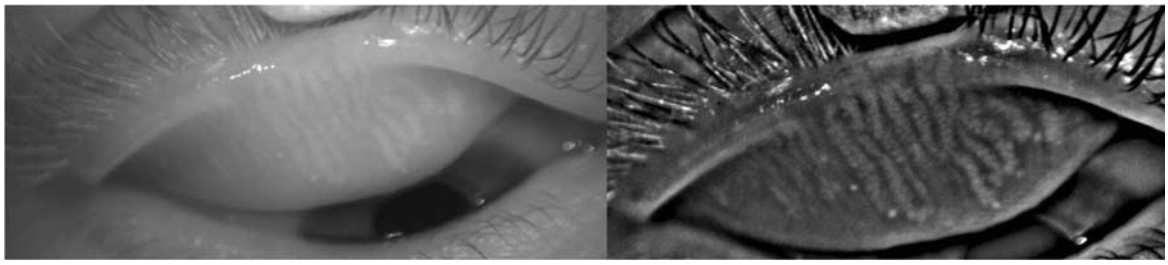


Figure 1-10: The entire superior palpebral surface could be imaged using the OCULUS Keratograph 5M (left). A processed image of the same eyelid highlights the MGs to make them more visible (right).

Non-contact meibography has been used to study MG duct distortion in patients with perennial allergic conjunctivitis,[249] MG dropout in patients with CL wear,[173] the difference in dropout between inferior and superior eyelids[250] and impact of age on MG dropout.[244] This method was also used to study the reliability of meibography grading scales,[241] the diagnostic parameters for obstructive and seborrheic MGD,[251,252] and the difference between obstructive MGD and aqueous deficient DE.[253]

Recently, TearScience has upgraded the LipiView to the LipiView II.[254] One of its new functions is meibography that features "Dynamic Meibomian Imaging™" technology in which adaptive transillumination is combined with dynamic illumination to yield high contrast images of the MGs.[254]

Despite the different methods available to undertake meibography, they all provide a gross view of the MGs and allow observers to make observations and monitor MG dropout and morphological distortion. More detailed information about MGs and MGD can be obtained by examining them under higher magnification. Previously, studying the ultrastructure of MGs required preparing and viewing histological sections belonging to animal models or from human exenterations,[236,255,256] but with advances in imaging technology it is now possible to view the ultrastructure of human MGs in vivo using a variety of anterior segment observational instruments.[257-263]

1.4.2.5.4 Confocal Microscopy

The Heidelberg Retinal Tomograph II/III with the Rostock Cornea Module (Heidelberg Engineering GmbH, Dossenheim, Germany) uses a 670nm LED light to provide high resolution scans (optical: 4µm horizontally, 2µm vertically, digital: 1µm/pixel vertically and horizontally) of the biological tissue of the anterior segment. The images generated at 384 x 384 pixels correspond to a 400x400µm field of view, and can be analyzed with the built-in software, or with ImageJ (Java software developed by National Institutes of Health).[258,264,265] The primary advantage of this technique is that it allows in vivo real-time viewing of MG acini structures in microscopic detail.

The first to report on observing MGs with confocal microscopy was perhaps Kobayashi et al.[260] in 2005, when they used confocal microscopy to observe the cells of the conjunctiva. The "web-like structures" seen below the conjunctival layers were presumed to be MGs.[260]

Meibomian gland acinar unit density (MGAUD), meibomian gland acinar longest and shortest diameter (MGALD, MGALSD) are two metrics that can be used in conjunction with the HRT internal software to assess the severity of MGD (Figure 1-11).[264,265] In addition, periglandular inflammatory cell density (ICD) can also be quantified.[264]

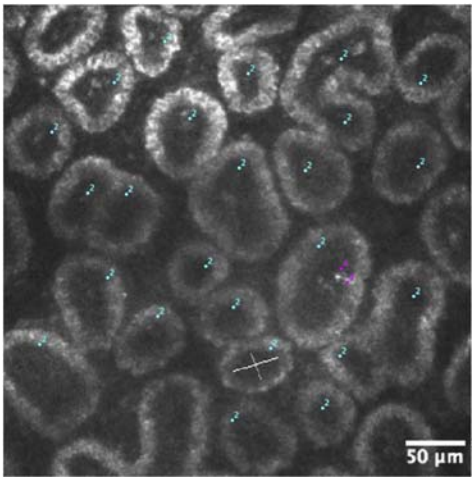


Figure 1-11: A 400µm x 400µm frame of acini clusters as seen with the HRT-II Rostock Cornea Module. Acini units (cyan) and inflammatory cells (magenta) are manually marked, and density for each is automatically calculated using the Cell Count® software (Heidelberg Engineering GmbH, Germany). Courtesy of Dr. Murat Dogru.

Confocal microscopy can assess the effectiveness of various MGD treatment strategies,[266] the effect of aging on conjunctival and MG structures,[267] the difference in morphology of gland structures between non-CL and CL wearers,[268] and reveal MG morphological changes in patients with SS.[269]

1.4.2.5.5 Optical Coherence Tomography

Imaging the MG structures with optical coherence tomography (OCT) for clinical management of dry eye is also not common, but has been shown to be feasible. Hwang et al. had used a fourier domain OCT with center wavelength of 1310nm (bandwidth = 100nm) to generate 3D images of MGs.[270] Images of MGs captured with OCT have greater detail than those observed by infrared meibography,[271] but do not surpass the theoretical limits of confocal microscopy.[272] Three dimensional image reconstructions of MGs have also been accomplished using a polarization sensitive OCT to study age-related changes to the MGs.[273]

1.4.2.5.6 Meibography Grading Scales

Currently, there has been no consensus on grading MG dropout, and thus several grading scales have been devised and used to assess MG dropout.

Mathers et al.[50]: The central 10 glands of the inferior tarsus were photographed and graded based on how much light was transmitted through the gland.

Jester et al.[236] found that more severe dropout corresponded with decreased transmission of light, due to increased thickness of the keratinized epithelium.

Grade 1: Normal gland

Grade 2: Gland visible with decreased absorption

Grade 3: Acini of gland severely atrophic with duct still visible

Grade 4: No gland structures visible

Den et al.[242] :

Grade 0: absent

Grade 1: present (more than half of lower lid)

Arita et al.[244] :

Grade 0: no dropout

Grade 1: dropout of less than <1/3 of total area of glands

Grade 2: dropout of more than 1/3, but less than 2/3 of total area of glands

Grade 3: dropout of more than 2/3 of total area of glands

McCulley et al.[54] and Aronowicz et al.[274,275]:

The central 7 glands of the tarsal plate were examined. Each gland is given a score from 0 to 4, where 0 represents no dropout, and 4 is complete dropout of that single gland. The score from each gland is summed up as a total out of 28. 0/28 would represent no dropout whereas 28/28 is complete dropout.

Shimazaki et al.[15] and Goto et al.[238,276]:

Grade 0: no dropout

Grade 1: loss of less than half the glands in inferior tarsus

Grade 2: loss of more than half the glands in inferior tarsus

McCann et al.[239]:

Grading was based on the percentage of glands that were absent. For example, if 50% of glands had dropped out, dropout would be graded as 0.5.

Pult et al.[245] :

> Using ImageJ to analyze photos, the area of dropout was subjectively defined and expressed as a percentage of the total area of the tarsal plate. The angles at which the glands are bent are also analyzed.

Srinivasan et al.[244,245,277] : indicated the presence or absence of white patches and gland tortuosity while using the grading by Arita et al. and ImageJ.

Ngo et al.[248] :

> Grade 0: 0% dropout
>
> Grade 0.5: 1 to 16% dropout
>
> Grade 1.0: 17% to 33% dropout
>
> Grade 1.5: 34% to 50% dropout
>
> Grade 2.0: 51% to 67% dropout
>
> Grade 2.5: 68% to 84% dropout
>
> Grade 3.0: 85% to 100% dropout

Currently there is no consensus on the number of increments that should be present in a grading scale for meibography. Bailey et al. has recommended that a scale of fine clinical sensitivity should not exceed one-third of the standard deviation of the discrepancy, using scales with smaller increments to increase the ability to detect clinical changes.[278,279]

1.4.2.6 Corneal and Conjunctival Staining

Corneal and conjunctival staining allows for the assessment of ocular surface integrity through the use of vital dyes. Despite its common use, the mechanism for damaged cells to take up the stain is still unknown.[280] The interpretation of corneal and conjunctival staining depends on the grading scale that is used. The staining is usually located at the inferior third of the ocular surface, representing the palpebral fissure where

desiccation occurs. Four ocular staining grading scales (Oxford scheme,[281] National Eye Institute-recommended system,[282] area-density combination index,[283] and the Sjogren's International Collaborative Clinical Alliance[284]) were compared by Sook and Park.[285] They found excellent inter-observer repeatability and reliability.[285] While these scales use subjective grading methods, automated and objective methods of grading also exist.[286,287]

1.4.2.7 Schirmer's Test

Schirmer's test is used to quantify tear and volume production. This is conducted by inserting a sterile standardized paper strip into the inferior lid margin. The strip is allowed to soak up tears for 5 minutes, and the amount of wetting represents the volume and production of tears. The test can be conducted with or without anesthesia of the ocular surface. The use of anesthesia (Schirmer II) reduces lacrimation input from the ocular surface and measures basal lacrimal secretion, and was reported to generally be more reliable.[288] There is currently no consensus as to where on the inferior lid margin the Schirmer's strip should be placed, which gaze direction to be held,[289] and whether the eyes should be open or closed.[290]

1.4.2.8 Lid Margin Evaluation

The eyelid margin is assessed using a biomicroscope. The eyelid margin may exhibit changes including the presence of irregularity,[231] erythema,[291] telangiectasia, and increased vascularity of the posterior margin. These signs are associated with MGD.[292] How these changes occur remains unknown. Some other important eyelid margin features that may be related to DE include the position of the Marx's line, and lid wiper epitheliopathy.

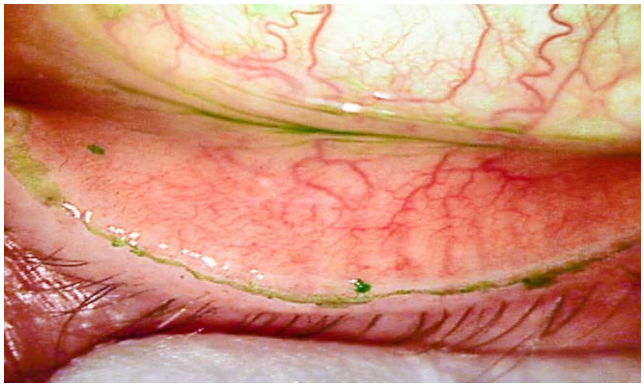


Figure 1-12: An irregular Marx's line with varying thicknesses is highlighted with lissamine green.

Marx's line is an anatomical feature, a demarcation representing the junction between the keratinized cutaneous skin of the face and the mucous epithelium of the conjunctiva. Marx's line is thought to originate from hyperosmolar stress at the leading edge of the peripheral tear meniscus, creating a region that is susceptible to vital dye staining.[293] In normal patients, Marx's line is straight, regular, and runs posterior to the MG orifices. In CL wearers[294] and MGD,[295] Marx's line appears to be irregular, thickened, and may shift anteriorly and bypass the MG orifices. Lissamine green can be used to stain and visualize Marx's line (Figure 1-12).[296] There are currently two different methods of grading Marx's line.

Kim et al.[294] grades of Line of Marx (LOM) as follows:

Grade 0: LOM mostly (>75%) posterior to the orifices

Grade 1: LOM mostly bisecting the orifices

Grade 1: LOM mixed posterior and bisecting the orifices

Grade 2: LOM mostly anterior

Grade 2: Mixed posterior, bisecting and anterior to the orifices

Grade 2: LOM mixed bisecting and anterior to the orifices

Yamaguchi et al.[295] grades Marx's line as follows:

Grade 0: the line runs entirely on the conjunctival side of the MG orifices

Grade 1: parts of the line arch forward to touch the MG orifices

Grade 2: the line runs through the MG orifices

Grade 3: the line lies on the skin side of the MG orifices

Yamaguchi grades the line on the central, nasal and temporal thirds of the eyelid, for a total score out of 9 per each eyelid.

The debridement, or the mechanical removal of debris using a golf club spud, of Marx's line was found to improve signs and symptoms in individuals with evaporative dry eye[297] and SS-DE.[298]

1.4.2.9 Lid Wiper Epitheliopathy

The lid wiper is an epithelial ridge located in both the superior and inferior eyelid, just posterior to the MGs. A number of different cell types make up this area (stratified epithelium, cuboidal cells, goblet cells, parakeratinized cells).[299] Without proper ocular surface lubrication, mechanical trauma from friction may induce cellular changes.[299] This could be observed with the instillation of ophthalmic dyes (Figure 1-13). A higher prevalence of lid wiper epitheliopathy was found in dry eye participants,[300] and correlated with dry eye symptoms.[178]

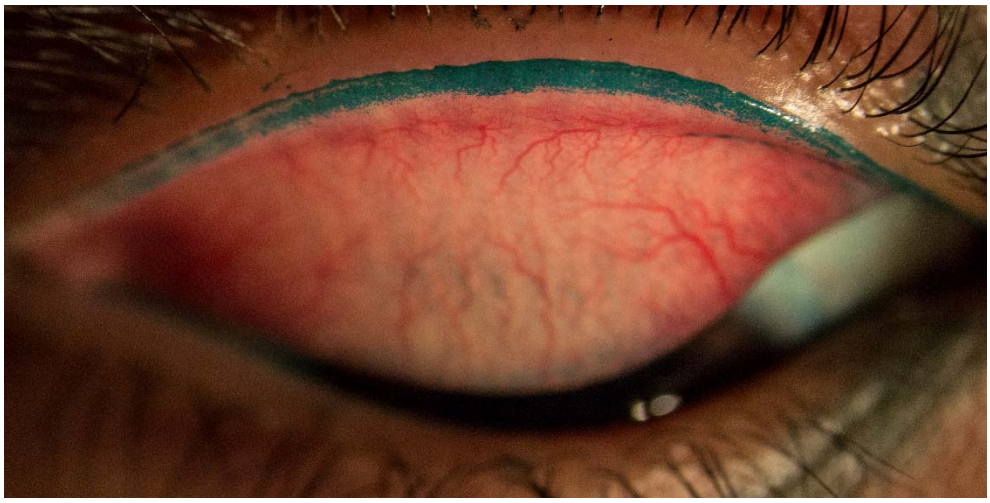


Figure 1-13: Lid wiper epitheliopathy can be observed with lissamine green. In this photo, the entire sagittal width of the lid wiper has been stained. Courtesy of Jalaiah Varikooty, Centre for Contact Lens Research.

1.5 Management of Dry Eye

1.5.1 Tear Supplementation

Artificial tears are a mainstay in DE management.[301] Artificial tears can range widely in composition and viscosity, [302] allowing them to target specific tear film or ocular surface deficiencies. For example, some artificial tears contain lipids[303,304] to supplement a deficient lipid layer, and some contain hyaluronic acid (a glycosaminoglycan) for water retention and to promote recovery of the ocular surface.[305,306] Some artificial tears have higher viscosities, allowing them be retained on the ocular surface for a longer period of time, but at the cost of visual quality.[307] Other artificial tears may contain a preservative-free formulation, which allow the drops to be instilled more frequently or used along-side other topical ophthalmic medication to relieve ocular surface disease.[308]

1.5.2 Warm Compresses

The delivery of heat (with or without mechanical pressure) to the eyelid comes in a variety of forms and can range from simple towel compresses, [309] to steam-emitting devices,[310] microwaveable heat-retaining bags[311] and electronically-controlled eye-mounted actuators.[312] The application of heat and pressure decreases the viscosity of meibum within the MGs, promoting increased delivery of meibum into the tear film.[313] Since heat is required to melt meibum, heat retention is an important aspect of therapy. Rapid dissipation of heat on the eyelid can be attributed to the dense vasculature of the skin,[314] where blood flow can quickly remove heat from the area. This may be counter-acted by applying some pressure around the eyelid to slow down blood flow.[314] Additionally, moistened towels tend to cool off quickly, therefore a device that retains heat well over the eyelids will be beneficial. A study of heat retention across several eyelid warming devices has been previous studied,[315] and there are currently multiple studies[310,311,316-319] that assess their efficacy in the treatment of MGD.

1.5.3 Omega-3 Fatty Acids

Omega-3 fatty acids (O3FAs) are essential fatty acids that can be acquired through a diet consisting of fish.[320] These oils play a role in modulating inflammation in many systemic diseases, such as bipolar depression,[321] diabetes mellitus,[322] and cardiovascular disease, [323,324] osteoarthritis,[325] and other autoimmune conditions.[326] Two types of O3FAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are incorporated into cell membranes for cell signalling and communication.[327] Specifically, O3FAs have antagonistic effects to pro-inflammatory Omega-6 fatty acids (O6FAs).[328] One such O6FA is arachidonic acid, a key molecule responsible for initiating the inflammatory cascade which results in the production of various inflammatory cytokines.[328] Various studies have found that a lower O3FA:O6FA ratio was not only associated with cardiovascular disease,[329-331] but also with a higher incidence of DE.[332]

Inflammation is a core component of DE.[333] Omega 3 fatty acid supplementation was shown to have an effect on the metabolic profile of the tear film,[334] and was found to improve various signs and symptoms in DE.[335-342] To-date, there is still no consensus as to the appropriate dosage for a therapeutic effect.[343] Some previous studies that reported successful DE outcomes have used at least 1000mg of combined EPA and DHA.[336,344-346]

1.5.4 Eyelid Hygiene

Microbial bioburden can adversely affect the ocular surface environment in a number of ways. Bacteria may use components of the tear film as food source,[347] thereby metabolizing and changing the tear film composition. The breakdown of meibum by staphylococcal lipases was previously confirmed.[348] Bacterial toxins can also trigger an immune reaction and exacerbate inflammation.[347] A common type of blepharitis is staphylococcal blepharitis, which can be diagnosed by observing the eyelashes for misdirection, eyelash loss, injection and/or the presence of matted/hard scales on the lashes.[349]

Another common type of blepharitis is *Demodex* blepharitis,[350] and this can be diagnosed by looking for waxy, cylindrical dandruff "cuffs" at the base of the eyelash (Figure 1-14).[351] A strain of bacteria (*Bacillus*

oleronius) isolated from *Demodex* may be responsible for triggering an inflammatory response within the surrounding tissue.[352,353]

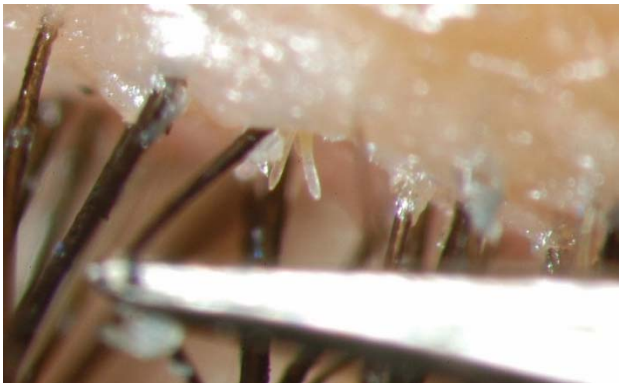


Figure 1-14: Two *Demodex* tails can be seen protruding from the lash follicle when the cylindrical dandruff is cleaned off and the lash is pulled aside.

Eyelid hygiene is a standard for the treatment of blepharitis.[354] The purpose of eyelid cleansing is to reduce microbial burden on the eyelids.[354] Decreasing microbial burden reduces the source of toxins and improves the environment of the ocular surface. Eyelid cleansing products can come in various forms. They may take the form of pre-moistened cotton pads,[355,356] foam-dispensing bottles,[357] spray bottles,[358] or an electronic rotary brush.[359] These products typically contain detergents or antimicrobial substances, and when combined with mechanical action, can be effective at removing bioburden and biofilms from the eyelid margins. Eyelid cleansing has been shown to improve symptoms of discomfort.[355,356]

Overall, DE is a multifactorial condition that requires a careful assessment of patient risk factors, symptoms and signs for an accurate diagnosis and management plan. There are a number of DE diagnostic tools and treatment strategies that have been recently developed, but their role in DE management is still unknown. Further investigation into these new technologies and treatments will allow researchers and clinicians to better understand their impact on DE disease.

2 Rationale

Dry eye (DE) is a multifactorial disease[1] that affects millions of individuals around the world.[2] This condition inflicts symptoms of ocular burning, stinging, and tearing, which can severely impact quality of life.[3]

The assessment of DE is conducted with a variety of clinical tests.[4] A thorough assessment consists of several aspects; a detailed history to evaluate DE risk factors, symptom assessment to evaluate subjective morbidity; and a battery of clinical tests to assess the function of the lacrimal functional unit and accessory glands.[4] One part of a routine DE assessment involves the examination of the meibomian glands (MGs). These glands produce and secrete meibum (lipids) into the tear film to reduce its surface tension, and reduce its rate of evaporation.[5]

One of several clinical tests that is used to examine the MGs is meibography. Meibography is a set of techniques that facilitates the viewing of MGs.[6,7] From viewing the MGs, the extent of MG atrophy can be observed and quantified. Meibomian gland atrophy is commonly graded using clinical grading scales, of which a wide variety exists.[8] There are numerous existing instruments that have been adapted to conduct meibography.[9-11] Chapter 3 explores some of the capabilities of MG imaging using infrared meibography, optical coherence tomography, and confocal microscopy. Currently, there is no consensus and set standards on MG imaging. A possible reason for this is that there is a current lack of sufficient evidence and knowledge to build these standards. One unknown aspect of grading MGs is whether meibography instruments can be used interchangeably (e.g. using instrument “A” on day 1, and instrument “B” on day 2). A second aspect is that the inter- and intra-observer variability for grading MG atrophy is not well studied. To address these knowledge gaps, Chapter 4 examines the repeatability and inter- and intra-observer variability when using the Keratograph 4 and K5M (OCULUS, Wetzlar, Germany) for MG imaging.

In addition to MG atrophy, two eyelid margin features (lid wiper epitheliopathy and Marx's line placement) have recently been associated with symptomatic DE[12] and meibomian gland dysfunction (MGD).[13] Mechanical trauma to the eyelid wiper region as a result of insufficient ocular lubrication has been hypothesized to cause DE symptoms in individuals with no other apparent DE signs,[14] and the anterior shift in Marx's line placement may be correlated with MGD.[13] To gain a better understanding of these two eyelid margin features, Chapter 5 investigates an age-matched female cohort to study these features in the context of symptoms and other DE signs.

New methods for the treatment of MGD are constantly emerging. Warm moist towel compresses have been a mainstay for MGD treatment, however they lack efficacy due to rapid cooling.[15,16] The MGDRx EyeBag (The EyeBag Company Ltd., Halifax, UK)[17] is a recently developed eyelid warming device that has been designed to retain heat longer than conventional warm moist towels. There is only one prior study that examined the efficacy of the EyeBag,[18] but that was based on a contralateral eye design, which made it difficult to accurately assess the impact of the device on symptoms. To address this limitation, Chapter 6 examines the efficacy of the EyeBag in a bilateral randomized controlled trial.

Lid debridement-scaling (LDS) is also a relatively newly reported clinical procedure, which functions to mechanically remove eyelid margin debris that obstruct the MG orifices.[19] This procedure was previously shown to be effective at improving MG function and symptoms in individuals with evaporative DE.[19] It is currently unknown whether this procedure could be extended to individuals with Sjögren's syndrome (SS), and how efficacious this procedure would be if used in such patients. To further understand the effectiveness of LDS, Chapter 7 examines the potential benefit of LDS in individuals with SS in a randomized controlled study.

The management of DE typically involves the use of artificial tears,[20] eyelid hygiene,[21] and supplementation with omega 3 fatty acids.[22,23] Although many studies examine the efficacy of each of these single components, very few have quantified the efficacy that can be achieved when all these products are used in combination. In Chapter 8, the efficacy of a combination of DE products is assessed over a duration of 3

months. Quantifying the effect of combined therapy will allow clinicians and researchers to further understand the impact of combination DE therapies.

In the concluding chapter, Chapter 9, a summary of the work and suggestions for future direction is presented.

3 Imaging Meibomian Glands using Optical Coherence Tomography and Confocal Microscopy

3.1 Overview

PURPOSE: To determine whether an experimental spectral domain ultra-long optical coherence tomographer (UL-OCT) can image meibomian glands (MGs) and to compare its acquired MG images with the Heidelberg Retinal Tomograph 3 (HRT3) with Rostock Cornea Module (RCM) *in vivo* laser scanning confocal microscope (Heidelberg Engineering GmbH, Heidelberg, Germany) and the Keratograph 5M (OCULUS, Wetzlar, Germany).

METHODS: Twelve healthy participants (7F, 5M) were enrolled in this study. The superior eyelids of participants were everted and imaged using the UL-OCT. Participants then had both the inferior and superior eyelids everted and imaged using the Keratograph 5M. Finally, the inferior eyelids were everted and imaged with the HRT3/RCM.

RESULTS and CONCLUSION: The UL-OCT was unable to image MGs. The HRT3/RCM imaged structures that resembled dermal rete pegs and papillae. Of the three methods used in this study, the only device that was able to successfully image MGs was the Keratograph 5M.

3.2 Introduction

The secretions of the meibomian glands (MGs) play a vital role in maintaining the stability of the tear film.[1] These modified sebaceous holocrine glands are located within the tarsal plate of the eyelids, and produce meibum (lipids) that travel out of the gland orifices at the eyelid margin, and into the tear film.[2] Meibum reduces the rate of tear film evaporation,[3] and functions to keep the tear film spread thinly without collapsing onto the cornea.[4] A lack of meibum delivery to the tear film could be due to several factors,[5] one of which is thought to be due to atrophy of the MGs.[6] With an unstable tear film, the ocular surface is repeatedly desiccated, which can potentially cause symptoms of ocular dryness, burning, and irritation.[7]

Imaging the MGs is a way to assess the severity of MG atrophy and it is facilitated with a group of techniques collectively known as meibography.[8] An overview of meibography techniques have been discussed elsewhere.[8,9] Generally, MGs can be observed by transilluminating the eyelid, or by illuminating the palpebral conjunctiva with infrared light.[10,11] The Keratograph 5M (OCULUS, Wetzlar, Germany) has a dedicated meibography function that use 840nm LED bulbs to illuminate the palpebral conjunctiva.[12]

Imaging the ultrastructure of MGs using confocal microscopy (CM)[13-23] and optical coherence tomography (OCT)[24-28] have also been reported, but their use in dry eye management is not as common, despite their wide range of use in medical applications.[29,30]

Optical coherence tomography images biological tissue *in vivo* in real-time[31] and is similar in principle to ultrasound imaging.[32] Instead of using sound energy, a low coherence light source is directed at biological tissue and a computer interprets the reflecting light by comparing it against a reference beam.[32] Various structures at different depths generate unique interference signals and a computer uses this information to calculate the depth of the structures.[32] The axial resolution of an OCT is highly dependent on the wavelength and bandwidth of the light source.[32] A shorter wavelength is more resolving, but a longer wavelength penetrates deeper tissue.[32] A larger bandwidth yields shorter coherence length and higher axial resolution.[32] A reconstructed image of MGs imaged with OCT is displayed in Figure 3-1.[26] Optical coherence tomography transverse sections of the superior eyelid have revealed that MGs are approximately 0.45mm in width, and are located approximately 0.4mm below the apical surface of the palpebral conjunctival epithelium (Figure 3-2).[25]

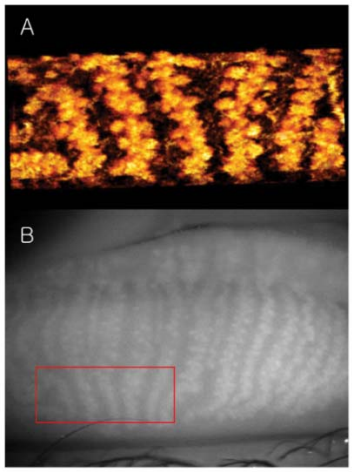


Figure 3-1: A) Meibomian gland imaged from an FD-OCT, compared with B) the infrared meibography image. Image from: Hwang HS, Shin JG, Lee BH, Eom TJ, Joo CK. In Vivo 3D Meibography of the Human Eyelid Using Real Time Imaging Fourier-Domain OCT. *PLoS One.* 2013;8(6):e67143.

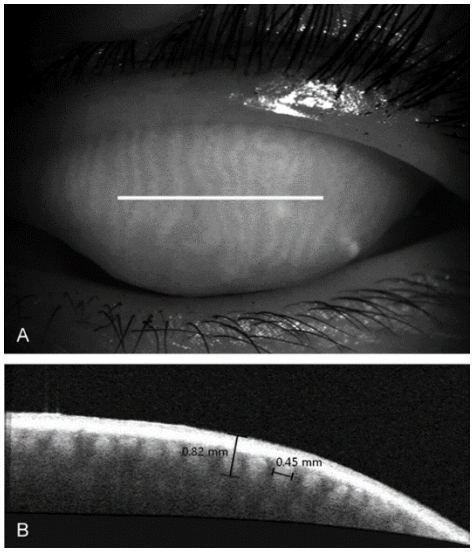


Figure 3-2: A) Infrared meibography image is compared to B) a transverse section of the superior palpebral conjunctiva. The white line indicates the location of the section. Image from: Hwang HS, Park CW, Joo CK. Novel noncontact meibography with anterior segment optical coherence tomography: Hosik meibography. *Cornea.* Jan 2013;32(1):40-43.

Confocal microscopy (CM) differs from OCT in that CM does not depend on interferometry to image axial structures.[32] Instead, scattering and reflectance of the laser by the specimen provides information at the plane of focus.[33] The depth of field in confocal microscopy is shallow and utilizes pinholes along the optical path to attenuate non-planar photon noise.[33] Although CM can generally achieve higher resolution scans than OCT,[32] one limitation is that tissue depth penetration is very limited.[34] An image of MG acini taken with CM is displayed in Figure 3-3.[17]

The Centre for Contact Lens Research (CCLR) have an experimental custom-built spectral domain ultra-long OCT (UL-OCT) (University of Waterloo, Ontario, Canada) that was used to successfully fit scleral lenses[35] and study various tear meniscus parameters.[36] It is currently unknown whether or not the UL-OCT is capable of imaging tarsal plate structures, in particular the MGs.

The purpose of this study was to determine whether the UL-OCT was capable of imaging MGs, and to compare them to images captured by the Heidelberg Retinal Tomograph 3 (HRT3) with Rostock Cornea Module (RCM) *in vivo* laser scanning CM (Heidelberg Engineering GmbH, Heidelberg, Germany), and the Keratograph 5M.

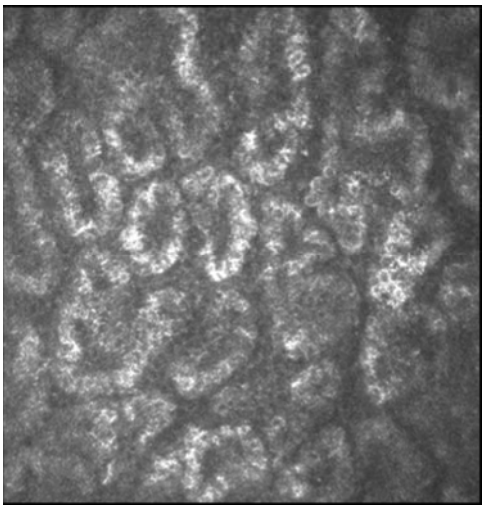


Figure 3-3: Meibomian gland acini structures from a healthy 26 year old male obtained using the HRT3/RCM confocal microscope. Image from: Fasanella V, Agnifili L, Mastropasqua R, et al. In Vivo

Laser Scanning Confocal Microscopy of Human Meibomian G lands in Aging and Ocular Surface Diseases. *Biomed Res Int.* 2016;2016:7432131.

3.3 Methods

The study was conducted at the Centre for Contact Lens Research at the University of Waterloo. The study was conducted in conformance with the ethical principles of the Declaration of Helsinki, the ICH guidelines for Good Clinical Practice, and the UW Guidelines for Research with Human Participants. Informed consent was obtained from all participants prior to enrollment in the study. Ethics clearance was obtained through a UW Research Ethics Committee prior to commencement of the study.

3.3.1 Participants

This was a single-visit study that enrolled 12 healthy participants (7 female, 5 male). To be eligible for the study, participants indicated that they were willing to undergo eyelid eversion and must not have worn contact lenses for at least 12 hours prior to the study visit. This was to minimize the occurrence of any inflammatory events.

3.3.2 Ultra Long Optical Coherence Tomography

The superior eyelid of the right eye was everted and imaged using the UL-OCT (Figure 3-4). The specifications of the UL-OCT and the settings used for imaging the meibomian glands can be found in Appendix 1. The area for scanning was selected to be at the horizontal midpoint, and one-quarter from the everted eyelid margin. The focal plane of the UL-OCT was adjusted so that the transverse section of the palpebral conjunctiva was clearly visualized. A horizontal scan capturing 30 images across an area of 4.96mm x 4.93mm was initiated using a foot pedal. This was repeated for the left eye.

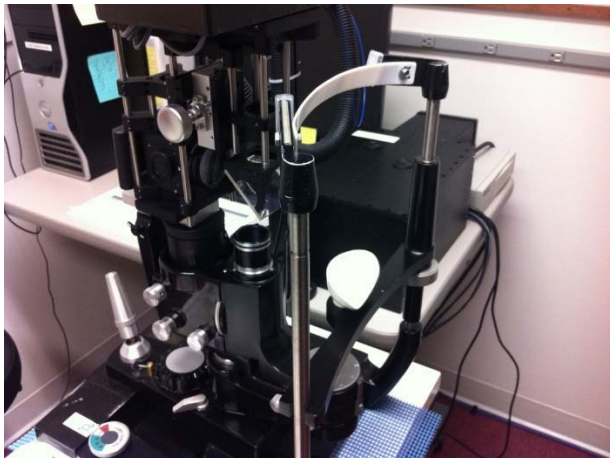


Figure 3-4: The UL-OCT is mounted onto a slit lamp platform, which allowed participants to remain stable during eyelid eversion.

3.3.3 Infrared Meibography

Participants were then imaged on the Keratograph 5M. The inferior and superior eyelids of the right eye was everted using a cotton-tipped applicator and imaged using the meibo-scan tool. This involved using infrared light-emitting diodes (LEDs) at a wavelength 840nm to illuminate the everted eyelids.[12] This was repeated with the left eye. Meibomian gland dropout of each eye was graded by using the grading method described in Arita et al.[11] (Grade 0: No dropout, Grade 1: up to 1/3 of the lid, Grade 2: 1/3 to 2/3 of the lid, Grade 3: More than 2/3 of the lid). Meibomian gland dropout was reported as an average between the two eyes.

3.3.4 Confocal Microscopy

The lower eyelids of each participants were then imaged using the HRT3/RCM.[37] A drop of proparacaine hydrochloride ophthalmic solution 0.5% (Alcaine, Alcon Canada, Mississauga, Ontario, Canada) was instilled in both eyes. An optical coupling gel (GenTeal, Novartis, Dorval, Quebec, Canada) was applied between the microscope lens and the Tomocap. The imaging plane of the HRT3/RCM was zeroed at the anterior surface of the Tomocap. Another application of the coupling gel was applied to the anterior surface

of the Tomocap. Participants were asked to position their eyes in superior gaze while the right inferior eyelid was everted. The Tomocap was applanated onto the surface of the palpebral conjunctiva. The focus plane was adjusted using the fine focus knob until MG structures could be visualized. A volumetric scan was conducted to obtain a tomogram of the region of interest. This was repeated with the left eye. The full specifications of the HRT3/RCM is listed in Appendix 2.

3.4 Results

The mean±SD age of the participants (7F, 5M) was 36±13 years old, ranging from 20 to 56 years old.

3.4.1 Infrared Meibography

Meibomian gland images were successfully obtained in all participants. A typical image of the MGs imaged with the Keratograph 5M is shown in Figure 3-5. The mean±SD meiboscore of this sample was 1.5 ± 1.4.

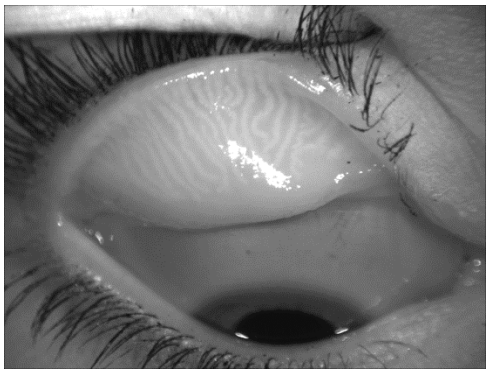


Figure 3-5: An image of the meibomian glands captured with the Keratograph 5M. Meibomian glands appear as an array of bright threads that vertically traverse the eyelids.

3.4.2 Ultra Long Optical Coherence Tomography

The images generated by the UL-OCT were 2048 x 2048 pixel slices, with 72dpi resolution. The instrument was unable to penetrate deep enough to detect the MGs. Figure 3-6 is a typical transverse section generated from a UL-OCT scan. ImageJ[38] was used to adjust brightness and contrast to bring out details of the glands, but was unsuccessful.

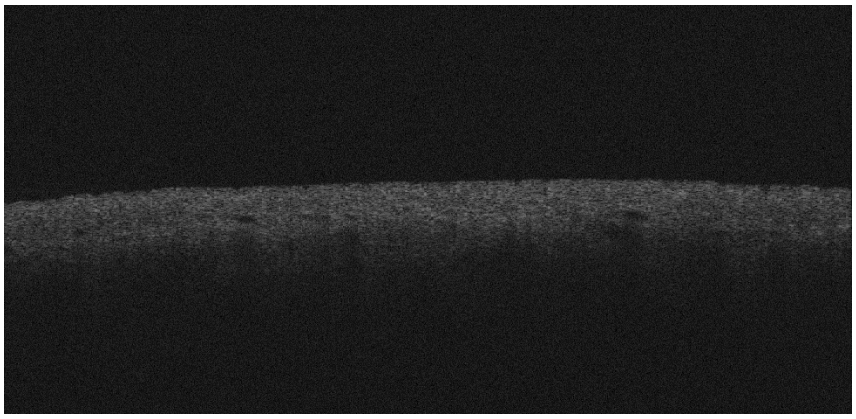


Figure 3-6: Typical UL-OCT of a transverse section of the superior palpebral conjunctiva. The horizontal width of the image corresponds to a length of 4.96mm. No meibomian glands could be detected.

3.4.3 Confocal Microscopy

The images generated from the HRT3/RCM were 384 pixel x 384 pixel slices, which corresponded to a 400μm x 400μm field of view. The manipulation and eversion of the eyelid induced tissue strain and distortion. This made it very difficult to maintain stability for imaging, and also made it impossible to determine the precise location and depth of the imaging plane. However, the observed structures appeared to be very superficial, located immediately below the basal epithelium of the eyelid margin, at a depth between 10μm - 100μm. The CM images of presumed MG acini structures were divided into two categories. The structures were round with either a hyper-reflective border or a hypo-reflective border. Four of the 12 participants had hyper-reflective circular structures, 7 participants had hypo-reflective circular structures, and images could not be obtained from the one remaining participant. Figure 3-7 contrasts and compares the two different types of circular structures that were observed in this study.

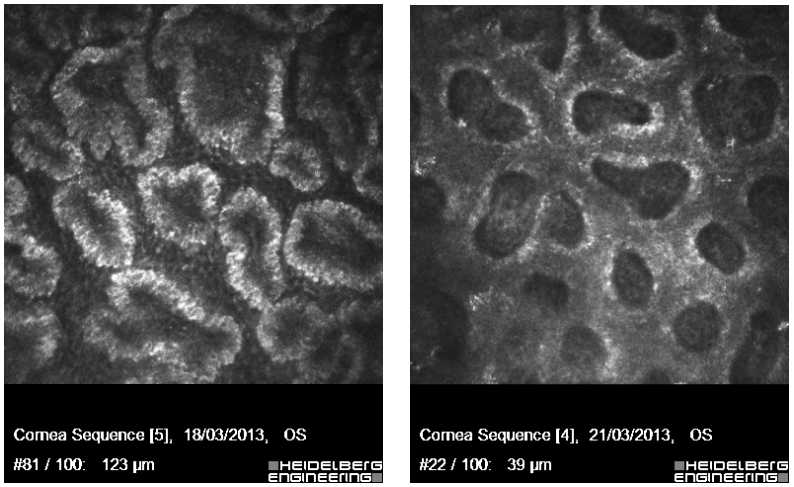
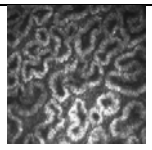
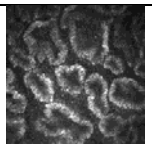
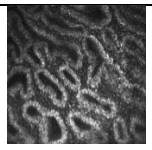
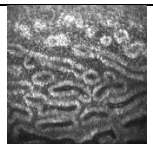
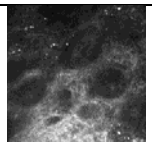
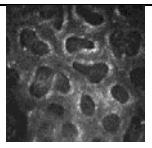
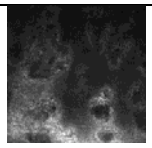
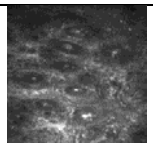
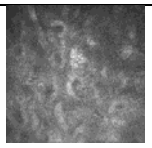
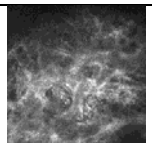
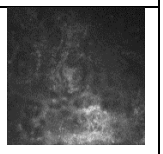


Figure 3-7: Left, HRT3/RCM image of presumed MG acini structures from a 20 year old Asian male. Hyper-reflective circular structures could be observed. Right, HRT3/RCM image from a 25 year old Caucasian female. Ringed structures were also present, but the borders were relatively hypo-reflective.

The hyper-reflective structures were observed only in participants with darker pigmented skin and the hypo-reflective structures were observed only in participants with lighter skin. Table 3-1 compares these structures across heavily-pigmented (HP) and lightly-pigmented (LP) skin types.

Table 3-1: Representative confocal images from participants with HP or LP skin types.

HP							
LP							

Acini structures that were previously described as "age-related atrophic changes" in a separate publication[18] were observed in relatively young participants in this study. These "age-related atrophic changes" were found in a 30 year old male with extensive MG atrophy (Figure 3-8), and also in a 25 year old female, with

relatively little MG atrophy (Figure 3-9). It appeared in the sample examined in this cohort that these "age-related atrophic changes" were independent of age and extent of MG atrophy.

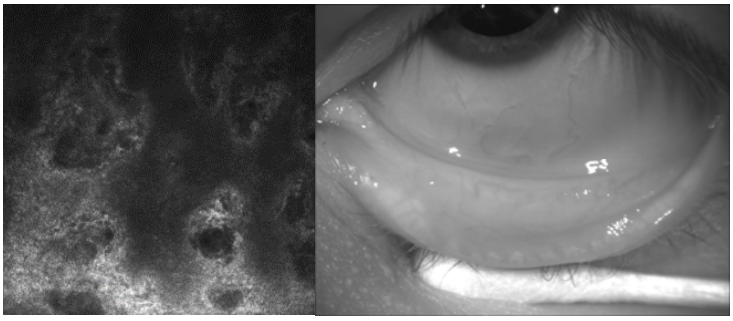


Figure 3-8: Left, presumed age-related atrophic structures imaged with HRT3/RCM. Right, Keratograph 5M infrared meibography image of atrophic meibomian glands in a 30 year old male.

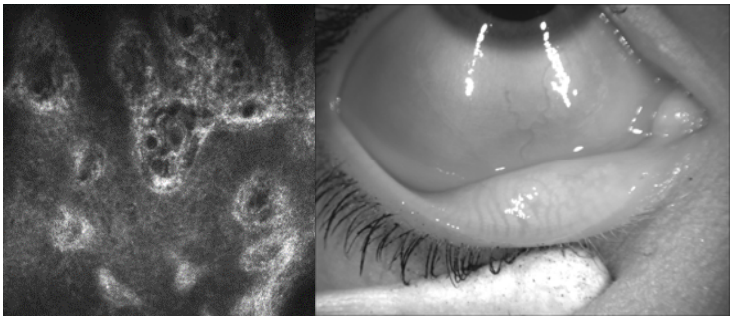


Figure 3-9: Left, presumed age-related atrophic structures imaged with HRT3/RCM. Right, Keratograph 5M infrared meibography image of intact meibomian glands in a 25 year old female.

A volumetric scan from one participant consisting of 40 images over a depth of 80 µm was stacked and stabilized using the Template Matching and Slice Alignment (TMSA) plugin[39] for ImageJ. The TMSA plugin consisted of two functions, the first (*cvMatch_Template*) allowed the user to select a region of interest (ROI) and the software would search the entire image stack that resembled the ROI. The second function (*Align_stack)* aligned the stack of images centered at the ROI.[39]

The resulting tomogram showed how the morphology of the MG acini changed with depth. Figure 3-10 is a montage view of the stacked and stabilized tomogram. Note that signal loss began very early on at a depth

of approximately 30μm (Figure 3-10) into the section. Beyond this depth, the laser was not able to penetrate any deeper into the tissue.

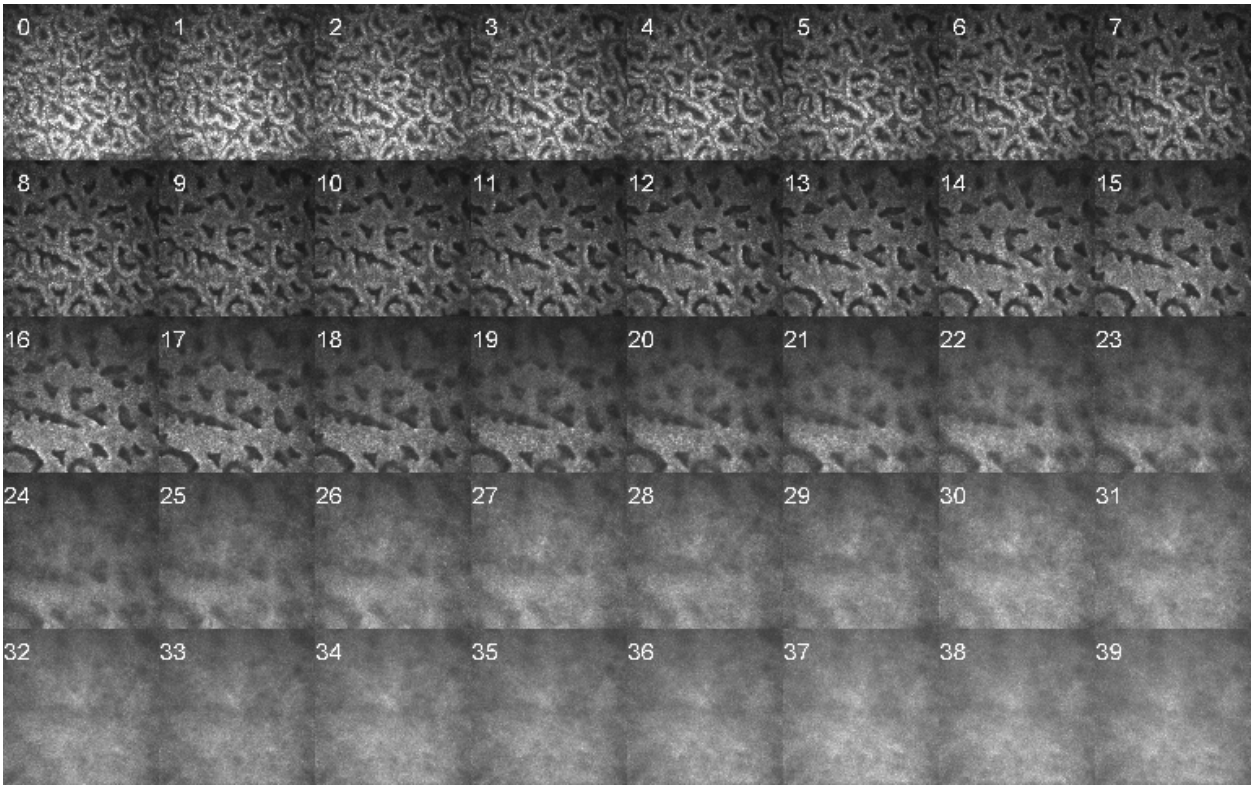


Figure 3-10: A montage of a 400 μm x 400 μm x 80 μm tomogram of the eyelid margin, starting from just below the basal epithelium of the epidermis. Each subsequent slice is 2μm deeper than the previous slice. The presumed MG structures merged together as depth increased. Signal to noise ratio depreciated considerably after 30 μm (slice 16).

3.5 Discussion

The UL-OCT was unable to obtain images of MGs and there may be a number of reasons why this was the case. A possible explanation is that the wavelength of 840nm may not have been able to penetrate through the tarsal plate deep enough to yield MG structures. The incident photons may have been scattered or absorbed. In either case, the lack of spatial and coherence information returning to the OCT detector means that no data could be used to generate depth information. A previous discussion on the relationship between

wavelength and penetration depth in a separate study[40] essentially stated that longer wavelengths are less attenuated than shorter wavelengths in deeper tissue. All previous studies that were successful in imaging meibomian gland structures using an OCT had used a wavelength of least 1300nm.[26-28] Furthermore, a recent study showed that OCT was able to detect acini structures in areas of the eyelid that was not detectable with infrared meibomian gland imaging,[41] suggesting that missing or truncated glands seen in infrared imaging could be misinterpreted as MG atrophy.

If the wavelength of 840nm was not appropriate for imaging MGs, then it may be interesting to investigate why the Keratograph 5M (also with 840nm light) was able to image the MGs. The reason may be based on the difference of the two imaging technologies. The Keratograph 5M was able to image the glands because the infrared detector depended on MG tissue light scattering to "see" the physical form of the gland. If the MG tissue was non-scattering and purely reflective, then the MGs would act as a mirror and appear as the LED light source illuminating them. Using an analogy, consider the reason why the moon is visible at night. The Moon is a matte object that scatters the photons of the sun in every direction and allows our eyes to receive the photons and discern the details of the moon. Similarly, MGs scatter light that allow the infrared detector to see the MGs. However, for interferometric-based imaging technologies such as the OCT, photons deflected off tissue must return on a certain path (spatial) and must contain useful interferometric information (coherence) for the computer to process depth information. Excessive scattering of photons in this case becomes detrimental to signal detection. This may explain why photon scattering in MG tissue allowed one type of technology to image MGs whereas another could not.

The images of MG acini structures imaged using the HRT3/RCM in this study closely resembled structures that appeared in various publications claiming to be MGs.[19,21] However, there were physical features that these structures exhibited that may indicate that they were not MG acini at all. Whether or not they were truly MG acini or not is worthy of discussion.

The CM images of MG acini depicted in the literature were often round and with bright or dark borders. This characterization is also consistent with cross-sections of dermal rete pegs. The rete pegs are finger-

like projections that anchor the epidermis to the dermis.[42] A cross-section of these rete pegs would thus yield a field of round structures. A cross-sectional histology and CM study of the eyelid by Knop et al.[43] described the presumed MG structures to be rete pegs and skin papillae (Figure 3-11). Furthermore, the previously mentioned "age-related atrophic changes"[18] were considered to be goblet cells imbedded within the epithelium.[43]

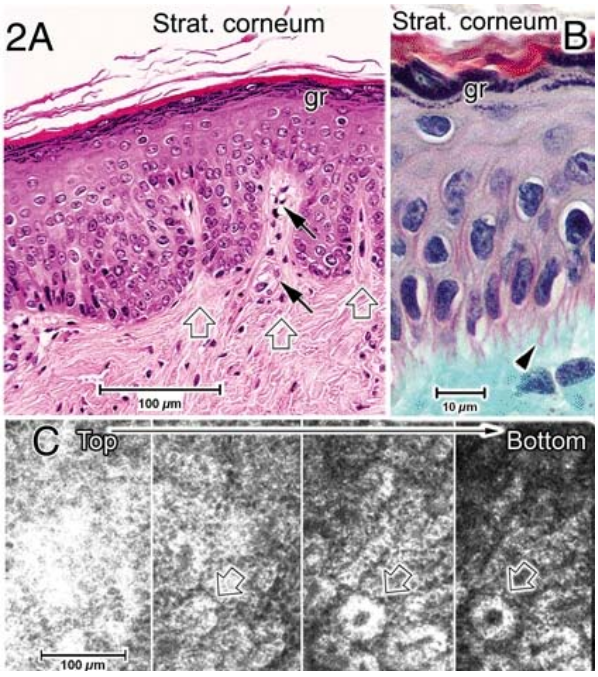


Figure 3-11: Rete pegs and papillae form undulating, finger-like projections into the epidermis (2A). A cross sectional view yielded bright circular structures as depth increased (2C). Image from Knop E, Knop N, Zhivov A, et al. The lid wiper and muco-cutaneous junction anatomy of the human eyelid margins: an in vivo confocal and histological study. *J Anat.* Apr 2011;218(4):449-461.

A separate dermatology article using CM to image skin nevi lesions showed that they contain circular features very similar to the questionable MG structures.[44] It appeared that the hyper-reflective border observed surrounding these structures may be related to pigmentation of skin – since it was only in pigmented skin that these hyper-reflective borders were observed. Meibomian gland epithelial cells were

not known to express melanin and the only case that reported on MG pigmentation was a case related to cosmetics contaminating the MGs.[45] Figure 3-12 shows a CM section of a nevus lesion.

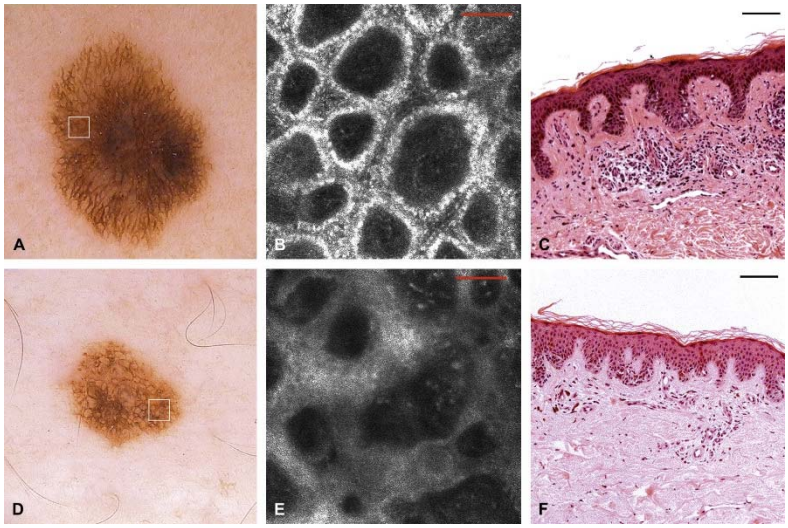


Figure 3-12: Confocal microscopy of the nevus yields hyper-reflective circular structures that are similar to the presumed MG structures. Image from: Pellacani G, Scope A, Ferrari B, et al. New insights into nevogenesis: in vivo characterization and follow-up of melanocytic nevi by reflectance confocal microscopy. *J Am Acad Dermatol.* Dec 2009;61(6):1001-1013.

To settle the discussion, a HRT3/RCM scan of the skin on a volunteer's forearm was obtained and it also appeared to be very similar to the presumed MG structures (Figure 3-13).

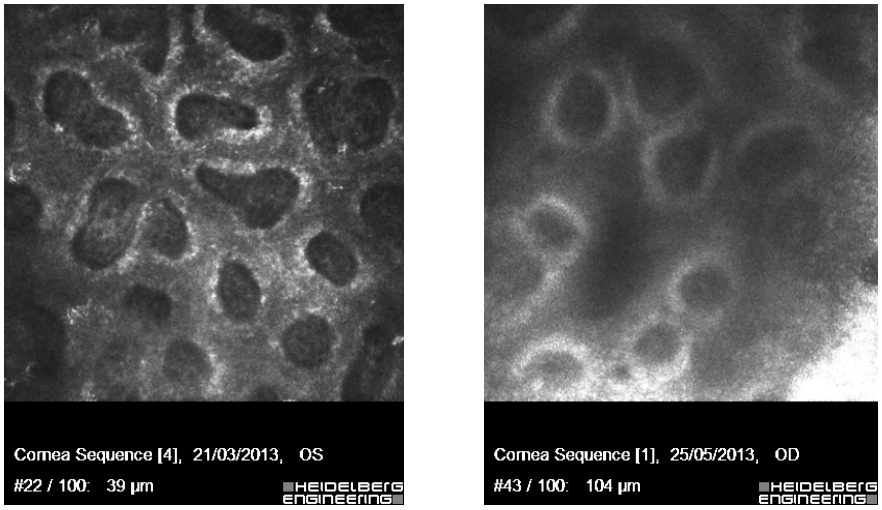


Figure 3-13: Left, presumed MG structures from the eyelid. Right, an image obtained from imaging skin on forearm of a pigmented individual.

Finally, the montage (Figure 3-10) showed how the shape of these presumed MG structures changed as a function of depth. As tissue depth was increased, the structures appeared to merge together and it could be seen that these structures do not at all resemble MGs.

3.6 Conclusions

The experimental UL-OCT was unable to obtain images of meibomian glands. We propose that the HRT3/RCM images of MGs were actually rete pegs and papillae of the dermis that were enhanced with the deposition of melanin. Of the three imaging devices used in this study, only the Keratograph 5M was able to successfully image the meibomian glands.

4 Repeatability of Grading Meibomian Gland Dropout using Two Infrared Systems

This chapter is published as follows:

Ngo W, Srinivasan S, Schulze M, Jones L. Repeatability of grading meibomian gland dropout using two infrared systems. *Optom Vis Sci.* Jun 2014;91(6):658-667.

	Concept & Design	Recruitment	Acquisition of Data	Analysis	Write-up/publication
Ngo	Y		Y	Y	Y
Srinivasan	Y		Y		Y
Schulze	Y			Y	Y
Jones	Y				Y

Table detailing role of each author in this publication (Y denotes significant contribution).

4.1 Overview

PURPOSE: To determine the inter/intra-observer repeatability in using the OCULUS Keratograph 4 (K4) and 5M (K5M) to grade meibomian gland (MG) dropout using meibography grading scales.

METHODS: The inferior and superior eyelids of 40 participants (35F, 5M, mean age 32yrs) were imaged 3 times each on both instruments. The images were split into one training and two study sets; the latter were graded (4-point meibography scale) by two observers on two separate occasions (24hrs apart) to determine repeatability. Semi-objective quantification of percentage MG dropout was conducted using ImageJ on K4 and K5M images. A finer 7-point meibography scale was used to grade a separate set of K5M images.

RESULTS: For the 4-point scale, inter-observer mean difference (MD) was 0.08 ± 0.55 on day 1 and 0.13 ± 0.50 on day 2, and the concordance correlation coefficient (CCC) was 0.79 and 0.81 on days 1 and 2 respectively. Intra-observer MD was 0.04 ± 0.54, CCC = 0.79 for observer 1, and -0.09 ± 0.60, CCC = 0.74 for observer 2. For the 7-point scale, inter-observer MD was 0.05 ± 0.45, (CCC) = 0.89 on day 1 and 0.01 ± 0.41, CCC = 0.91 on day 2. Intra-observer MD was -0.10 ± 0.35, CCC = 0.93 for observer 1 and -0.06 ±

0.30, CCC = 0.95 for observer 2. Percentage dropout measured between the K4 and K5M images showed lack of agreement, with 21.8% coefficient of repeatability. There was no significant correlation ($r<0.2$; $p>0.05$) between meibography score and clinical signs (corneal staining, gland expressibility, telangiectasia, vascularity, lash loss), however there was a high correlation ($r=0.77$; $p<0.05$) between meibography score with percentage dropout.

CONCLUSION: Observers graded from -1 to +1 grade units between and within themselves for a 4-point scale, 95% of the time. Although the inter/intra-observer repeatability of the K4 and K5M were very similar, a high rate of disagreement in percentage dropout between K4 and K5M images suggests that the two instruments cannot be interchanged. MG dropout scores did not correlate significantly with clinical signs. Using a finer scale may be beneficial for detecting change.

4.2 Introduction

Meibomian glands (MGs) are modified sebaceous glands that are embedded within the tarsal plate. These glands consist of a long central duct surrounded by secretary acini units, with orifices terminating posterior to the mucocutaneous junction of the eyelid.[1] An oily secretion, termed meibum, is produced from these glands, which serves to stabilize the tear film and slow its evaporation rate.[2] Obstruction of these glands can cause changes to the MG tissue and lead to a decreased production or stasis of the oils within the glands. This results in a condition termed meibomian gland dysfunction (MGD).[3] The International Workshop on Meibomian Gland Dysfunction recommends MGD to be defined as:

"Meibomian gland dysfunction is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. This may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease."[3]

MGD is a condition with a reported prevalence ranging from 3.5%[4] to 69.3%.[5] This wide range in reported prevalence is due to a number of factors, including ethnic differences in study participants and the classification of MGD adopted for each study.[6] MGD is believed to be the leading cause of evaporative DE,[7] which is a major cause for contact lens discontinuation.[8] Some clinical tests used to diagnose and assess MGD include biomicroscopy of the eyelid margin,[9] observation of MG expressibility[10] and expressed meibum quality,[11] in conjunction with other tests such as tear film break-up time,[12,13] tear film lipid layer thickness,[13,14] symptom assessment,[10] and meibography.[3,15,16]

Meibography relates to the technique of imaging the MGs (either with infrared film[17] or digital capture[18]) and is a method that allows the appearance of the glands to be characterized. Traditionally, this technique was performed by everting the eyelids over a white light transilluminator, which revealed the MGs through the lid and allowed the examiner to view them.[16,19,20] With advancements in imaging technology, the more recent practice is to use infrared (IR) light to illuminate the everted eyelid to reveal the MGs for digital

capture with an IR-sensitive camera. The MGs can be physically characterized as an array of "string-like" structures that traverse the palpebral surface vertically.[21] Partial loss or truncation of these structures is presumed to represent MG "dropout" or atrophy. To assess the degree of MG dropout, a number of different clinical grading methods have been reported,[21-23] however the most appropriate procedure for tracking the course of dropout has yet to be determined.

The OCULUS Keratograph 4 (K4; OCULUS, Wetzlar, Germany) is primarily a corneal topographer that includes an IR camera (for pupillometry) that has been adapted for meibography.[24] The OCULUS Keratograph 5M (K5M; OCULUS, Wetzlar, Germany) is a more recent model that was developed to be used as a "true" meibography device. It has been optimized for meibography by increasing the field of view, modifying the position of the IR diodes to minimize interfering reflections, and generating post-processed images that highlight the MGs (Figure 4-1).

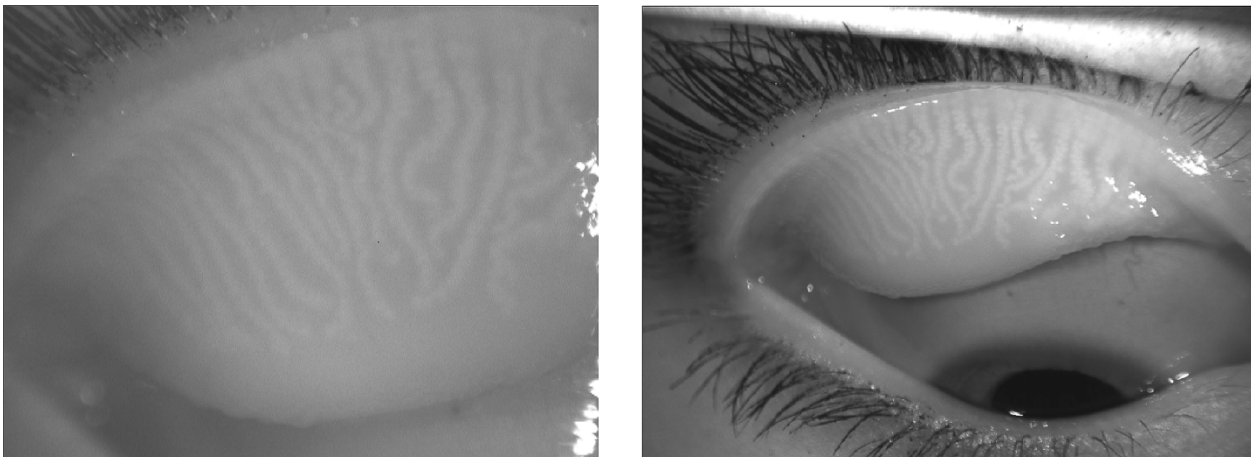


Figure 4-1: The K4 (left) and K5M (right) images show a marked difference in appearance. K5M images are brighter, show high contrast, and a larger field of view.

The purpose of this study was to assess the inter- and intra-observer repeatability of two observers when grading images obtained with these two instruments, to determine if they are interchangeable in a clinical setting, where patients may return often and see different practitioners or be imaged on either instrument. In addition, a grading scale with more scale steps is introduced, to investigate its effect on user repeatability.

4.3 Methods

Ethics clearance was obtained through the Office of Research Ethics at the University of Waterloo prior to commencement of the study and all procedures adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants prior to enrolment in the study.

4.3.1 Participants

Forty participants (35 female, 5 male, mean age = 32.2 ± 11.9 yrs, age range = 19 to 62) without any uncontrolled systemic conditions, and without any history of ocular disease in which lid eversion would cause unacceptable discomfort (i.e. recent blepharoplasty, refractive surgery) were enrolled in this prospective, single visit study. Participants were not permitted to wear contact lenses on the day of the appointment. Thirteen of the 40 were contact lens wearers.

The appointment consisted of a brief case history to obtain demographic information, followed by a visual acuity measurement and biomicroscopy of the anterior eye and eyelid margins. The following clinical parameters were recorded: corneal staining, eyelid telangiectasia, lash loss, vascularity, and degree of MG orifice obstruction of the inferior central 8 MG orifices. The scores pertain to the eye that was selected randomly for meibography. A table displays more information on the specific clinical techniques conducted (see Appendix 1, which is a summary of clinical tests and grading scales).

After a 10 minute break, meibography was conducted with both the K4 and K5M.

4.3.2 Meibography

The participant was seated and their head positioned comfortably on the K4. Both the inferior and superior eyelid of one randomly selected eye were imaged sequentially. The participant was then asked to withdraw their head from the instrument for approximately 5 seconds, and then repositioned their head on the K4 again, and images of the inferior and superior eyelid of the same eye were obtained again in an identical

fashion. This was repeated so that a total of 6 images were acquired (3 superior, 3 inferior). This process was repeated with the K5M.

A total of 480 images were acquired from the 40 participants. The images were sorted into 3 sets (each set contained 1 superior and 1 inferior eyelid image from both the K4 and K5M of all participants, for a total of 160); one set was used to train two observers, and the remaining two sets were presented to the same two observers for grading on two separate occasions (24 hours apart) to determine inter- and intra-observer agreement.

All images from each set were randomized and then sequentially presented to the two observers on a 50" high definition television screen, in a darkened room. Each image was displayed for 15 seconds. No communication between the two observers was permitted. MG dropout was assessed using both a subjective grading scale and a semi-objective computer based image analysis method.

4.3.3 Subjective dropout grading with a 4 point scale

The areas of partial or complete MG dropout of the upper and lower lids were assessed using the 4 point (0 – 3) grading scale described by Arita et al.[21] The grading steps are as follows: Grade 0: no dropout, Grade 1: less than 1/3 total area dropout, Grade 2: 1/3 to 2/3 total area dropout, Grade 3: more than 2/3 total area dropout. The grading number assigned is termed the "meiboscore".[21]

4.3.4 Semi objective digital grading

MG dropout from images used on the first day of subjective grading was digitally quantified using ImageJ 1.46r (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA; http://imagej.nih.gov/ij),[25] an open source image analysis software. A total of 160 images consisting of 80 K4 images, and 80 K5M images were ordered randomly. Areas of MG loss in those images were measured by manually outlining the regions on the everted eyelid where MGs were not detected. This value was then divided by the total area of the everted eyelid to calculate percentage area loss, as previously reported by Srinivasan et al.[24]

4.3.5 Subjective dropout grading with a 7 point scale

A second repeatability experiment was conducted using a 7 point scale. This scale was derived by adding half-steps to the Arita 4 point scale. The resulting scale steps were: Grade 0: 0% dropout, 0.5: 1 to 16%, 1: 17 to 33%, 1.5: 34 to 50%, 2.0: 51 to 67%, 2.5: 68% to 84%, 3.0: 85 to 100%. A set of 42 K5M (21 superior, 21 inferior) images were obtained from the Centre for Contact Lens Research (CCLR) archives. The images were selected by a third investigator and images were deliberately selected such that the set would contain a balanced distribution of MG dropout across the scale. The images were then presented to both observers for visual grading on two separate occasions (24 hours apart) to determine inter- and intra-observer agreement.

4.3.6 Statistical Analysis

Inter- and intra-observer repeatability was calculated using mean differences and concordance correlation coefficient (CCC). Grading repeatability for superior and inferior eyelids for each device were also calculated using mean differences and CCC. Concordance correlation coefficient evaluates how well a pair of observations fall along a 45 degree line from the origin.[26] To help interpret CCC values, McBride[27] interpreted CCC values as follows:

CCC < 0.90 = poor.

CCC 0.90-0.95 = moderate.

CCC 0.95-0.99 = substantial.

CCC > 0.99 = almost perfect.

Correlation of clinical signs to meibography scores was determined using Pearson's r. Paired t-test was used to determine percent MG dropout difference between K4 and K5M images analyzed with ImageJ. Data analyses were conducted using Statistica 7.1 (StatSoft Inc. Tulsa, OK, USA), MedCalc 12.3 (MedCalc Software bvba, Broekstraat 52, 9030 Mariakerte, Belgium), and GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA). Level of statistical significance was set at $p < 0.05$.

4.4 Results

4.4.1 Clinical outcomes

The clinical findings are reported in Table 4-1, and a summary of the meiboscores when using the Arita 4 point grading scale from the two observers over the two days are displayed in Table 4-2.

There was no significant difference ($p > 0.05$) in average meiboscore given by the two observers, nor over the two different days ($p > 0.05$) on which grading occurred.

The meiboscores from observer 1 on the first day from the K5M were used to calculate Pearson's correlations with the clinical signs. The results are displayed in Table 4-3. There were no statistically significant correlations between clinical signs and meiboscore, or clinical signs with percentage dropout (Pearson's $r < 0.2$; all $p > 0.25$). However, there was a high correlation between meiboscore with percentage dropout (Pearson's $r = 0.77$; $p < 0.05$).

Table 4-1: Summary of clinical test results.

Technique	Mean ± SD	Range
Corneal Staining	38.75 ± 77.09	0 – 400
Telangiectasia	0.100 ± 0.38	0 – 2
MG orifice obstruction (of the inferior central 8 orifices)	1.85 ± 1.00	0 – 4
Vascularity	1.05 ± 0.85	0 – 3
Lash loss	0.28 ± 0.55	0 – 2

Table 4-2: Summary of meiboscores (mean ± SD, medians and quartiles) from each observer on both days.

	Observer 1 Day 1	Observer 1 Day 2	Observer 2 Day 1	Observer 2 Day 2
Mean ± SD	0.77 ± 0.85	0.73 ± 0.83	0.70 ± 0.85	0.60 ± 0.81
Low	0	0	0	0
1st Quartile	0	0	0	0
Median	1	0	1	0
3rd Quartile	1	1	1	1
High	3	3	3	3

Table 4-3: Pearson's r correlation of clinical signs to meiboscore for observer 1 on day 1.

	Corneal Staining	Telangiectasia	MG orifice obstruction	Vascularity	Lash loss
Pearson's r correlation to meiboscore	r = 0.03 p = 0.87	r = -0.03 p = 0.85	r = 0.18 p = 0.26	r = -0.06 p = 0.71	r = 0.03 p = 0.83

Raw data of both observer 1 and observer 2 on both days are listed in a table (see table, Appendix 2, which is the raw grading scores for both observers on both days) and a histogram displays the grading distribution (meiboscores) for both observers on day 1 (Figure 4-2).

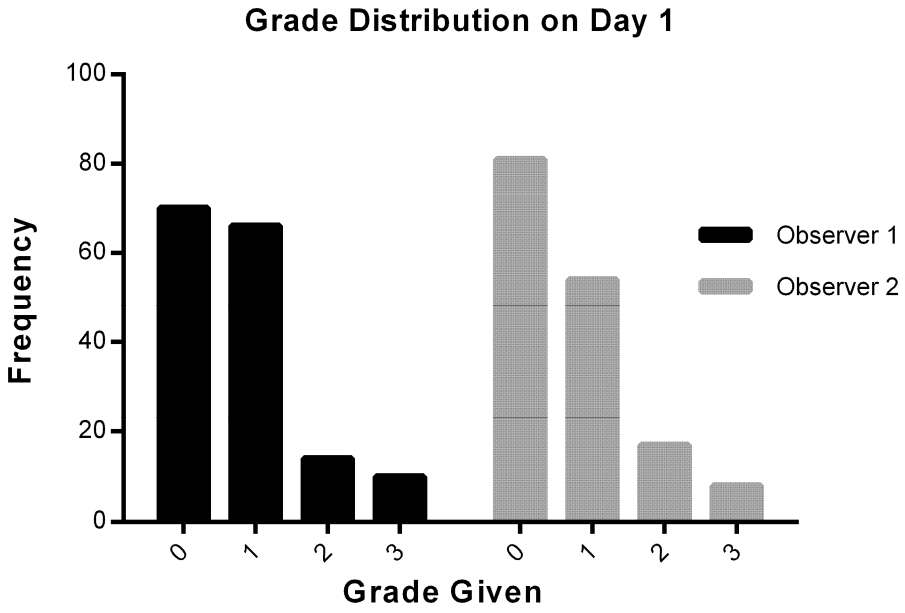


Figure 4-2: This histogram outlines the grading distributions of the 2 observers on day 1. A total of 160 images from the K4 and K5M were graded by two observers on day 1. The frequency of each grade assigned by each observer was tallied and summarized in this histogram. The possible grades for each image was 0 to 3 (for both the superior and inferior eyelid separately).

4.4.2 Inter-observer repeatability of grading with the Arita 4 point scale.

Limits of agreement plots for the inter-observer mean difference, with 95% upper and lower limits of repeatability between the two observers are shown in Figure 4-3. The 95% limits of agreement spanned from -1.00 to +1.16 on day 1, and -0.85 to +1.11 on day 2.

The inter-observer mean difference and CCCs on day 1 and day 2 were similar. On day 1, mean difference ± SD was 0.08 ± 0.55 grade units, with CCC = 0.79. On day 2, mean difference ± SD was 0.13 ± 0.50 grade units, with CCC = 0.81. There was an agreement of 110/160 (69%) images on day 1, and 121/160 (76%) images on day 2.

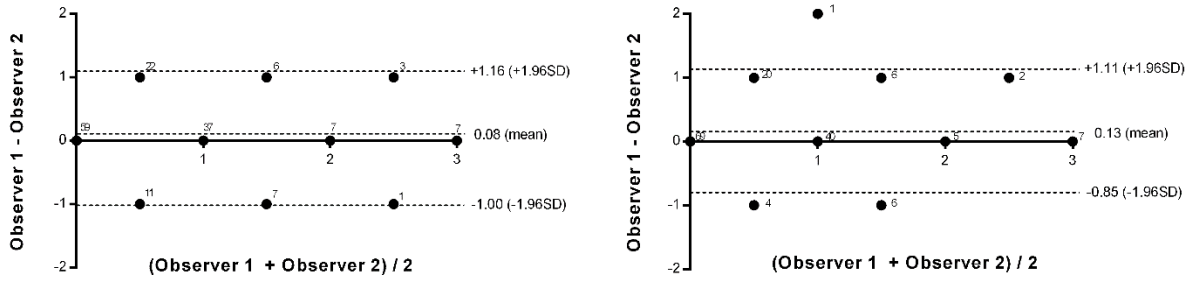


Figure 4-3: Data were pooled from both the K4 and K5M. Left: limit of agreement plot showing day 1 inter-observer repeatability. Right: limits of agreement plot showing day 2 inter-observer repeatability. Numbers at each point indicate the number of overlapping points.

4.4.3 Intra-observer repeatability of grading with the Arita 4 point scale.

Limits of agreement for the intra-observer mean difference were similar for observer 1 (95% upper and lower limits of agreement: -1.02 to +1.10) and observer 2 (-1.27 to +1.09). The limits of agreement plots are shown in Figure 4-4.

The intra-observer mean differences and CCC were also similar for both observers. The intra-observer mean difference ± SD for observer 1 was 0.04 ± 0.54 grade units, with CCC = 0.79. For observer 2, the intra-observer mean difference was -0.09 ± 0.60 grade units, with CCC = 0.74. There was an agreement of 116/160 (73%) images for observer 1 and 121/160 (76%) for observer 2.

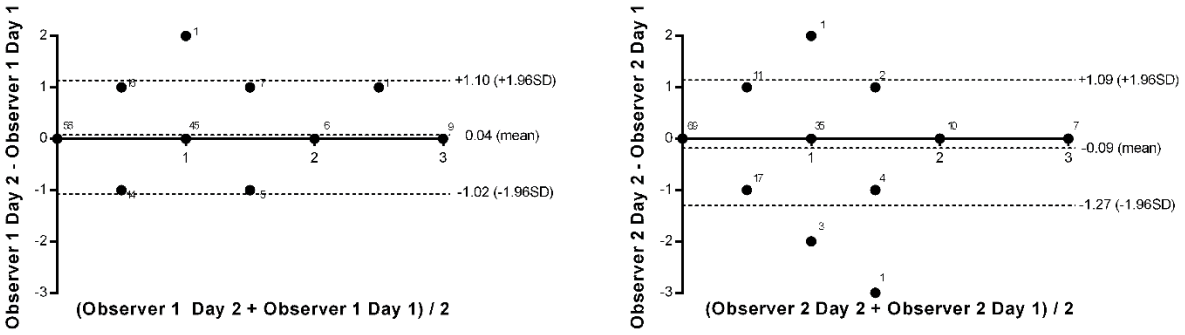


Figure 4-4: Data were pooled from both the K4 and K5M. Left: limit of agreement plot showing intra-observer repeatability of Observer 1 between day 1 and day 2. Right: limits of agreement plot showing intra-observer repeatability of Observer 2 between day 1 and day 2. Numbers at each point indicate the number of overlapping points.

The inter- and intra-observer mean differences ± SD and CCC for both observers, subdivided by device, is displayed in Table 4-4. The inter- and intra-observer repeatability when grading images from either instrument were similar, however, based on CCC scores there was slightly better intra-observer repeatability when the observers were grading images obtained with the K5M than with the K4. This slight difference is may not be clinically significant, as the intra-observer mean differences remained similar.

Table 4-4: Summary of observers' inter- and intra-observer mean differences ± SD grade units and CCC on each individual device when grading with the Arita 4 point scale.

K4				K5M			
Inter-observer		Intra-observer		Inter-observer		Intra-observer	
Day 1	Day 2	Observer 1	Observer 2	Day 1	Day 2	Observer 1	Observer 2
0.01 ± 0.58 CCC = 0.78	0.08 ± 0.52 CCC = 0.80	-0.08 ± 0.49, CCC = 0.76	-0.09 ± 0.51, CCC = 0.68	-0.16 ± 0.51, CCC = 0.79	0.18 ± 0.47, CCC = 0.81	-0.01 ± 0.85, CCC = 0.82	-0.10 ± 0.68, CCC = 0.80

4.4.4 Superior and inferior eyelid repeatability for the Arita 4 point scale

The limits of agreement for superior and inferior eyelids were separately analyzed on each device. Data from an arbitrary observer were used. On the K4, intra-observer mean difference with the 95% upper and lower limits of agreement, and CCC were 0.15 (-1.36 to 1.66), CCC=0.61 for the inferior eyelid, and 0.05 (-1.12 to 1.22), CCC=0.72 for the superior eyelid. On the K5M, intra-observer mean difference with the 95% upper and lower limits of agreement were 0.10 (-1.06 to 1.26), CCC=0.76 for the inferior eyelid, and 0.08 (-0.74 to 0.89), CCC=0.84 for the superior eyelid. In all cases, the grading of the superior eyelid was more repeatable than grading the inferior eyelid, and grading with the K5M was more repeatable than the K4. Figure 4-5 and Figure 4-6 compares the limits of agreement between superior and inferior eyelids conducted across the K4 and K5M.

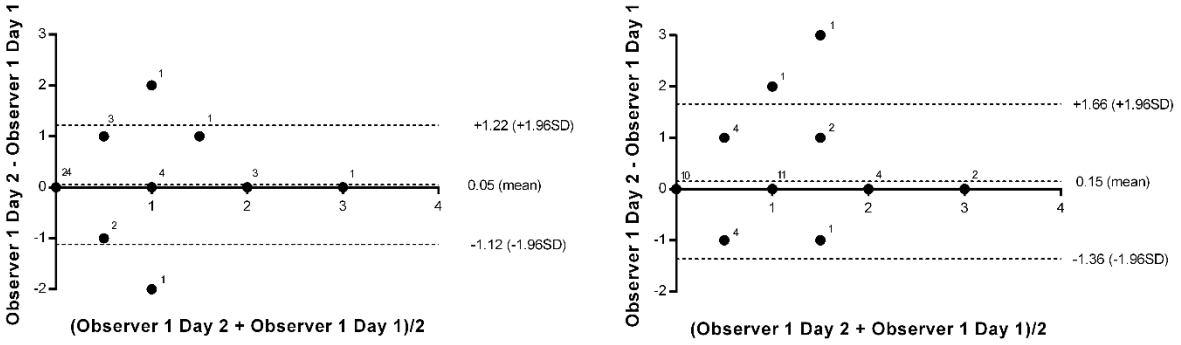


Figure 4-5: Left: limit of agreement plot showing intra-observer repeatability of observer 1 grading the superior eyelid with the K4. Right: limit of agreement plot showing intra-observer repeatability of observer 1 grading the inferior eyelid with the K4. Numbers at each point indicate the number of overlapping points.

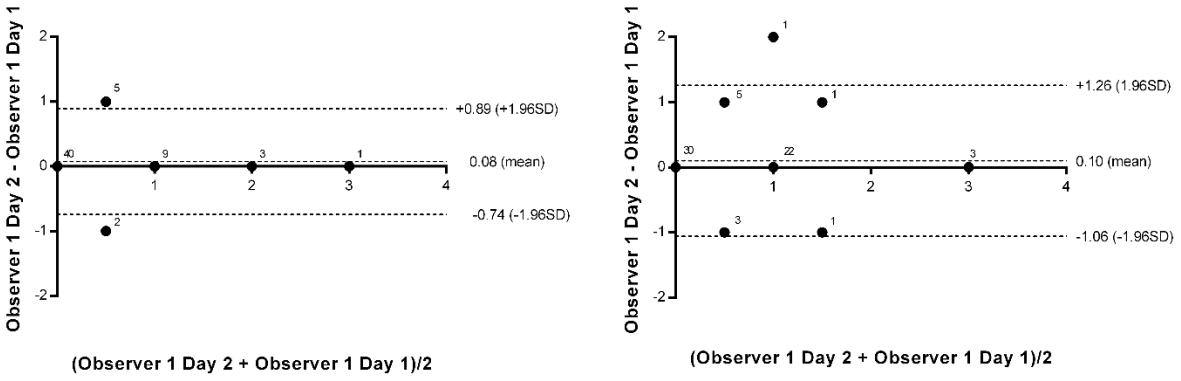


Figure 4-6: Left, limit of agreement plot showing intra-observer repeatability of observer 1 grading the superior eyelid with the K5M. Right, limit of agreement plot showing intra-observer repeatability of observer 1 grading the inferior eyelid with the K5M. Numbers at each point indicate the number of overlapping points.

4.4.5 Second repeatability experiment

As can be seen from the results presented above, the majority of images in this study belonged either to the Grade 0 or Grade 1 category, with a lack of images representing the higher end of the scale. Furthermore, the high rate of agreement between the two observers indicated that the scale used may be too coarse. These two issues were addressed by running a second repeatability experiment using a 7-point scale to grade a set of K5M images. The distributions of grades given by the 2 observers are shown in Figure 4-7.

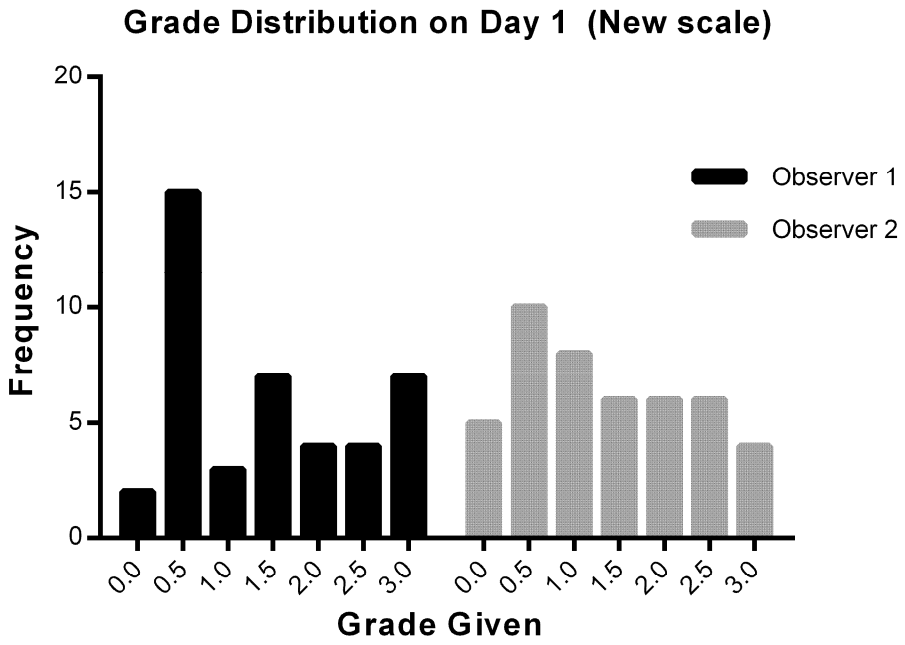


Figure 4-7: This histogram outlines the grading distributions of the 2 observers on day 1, using the new 7 point scale. A total of 42 K5M images were presented to the two observers for grading on day 1. The grades that each observer assigned was tallied and summarized in this histogram. The possible grades were from 0.0 to 3.0 in 0.5 steps (for both the superior and inferior eyelid separately).

On day 1, inter-observer mean difference ± SD was 0.05 ± 0.45 grade units with CCC = 0.89. On day 2, inter-observer mean difference ± SD was 0.01 ± 0.41 grade units with CCC = 0.91. The rate of perfect agreement was 22/42 (52%) and 24/42 (57%) on day 1 and day 2 respectively. The 95% limits of agreement spanned from -0.84 to +0.94 on day 1, and -0.78 to +0.80 on day 2 (Figure 4-8).

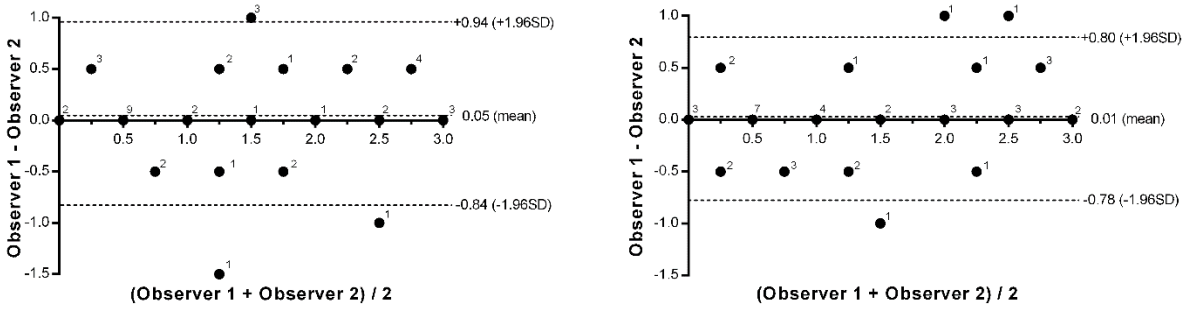


Figure 4-8: Left: limit of agreement plot showing day 1 inter-observer repeatability using the new 7 point scale. Right: limit of agreement plot showing day 2 inter-observer repeatability using the new 7 point scale. Numbers at each point indicate the number of overlapping points.

Intra-observer mean difference ± SD was -0.10 ± 0.35 grade units, with CCC = 0.93 for observer 1, and -0.06 ± 0.30 grade units, with CCC = 0.95 for observer 2. The rate of agreement was 26/42 (62%) for observer 1, and 27/42 (64%) for observer 2. The 95% limits of agreement spanned from -0.79 to +0.60 for observer 1, and -0.51 to +0.62 for observer 2 (Figure 4-9).

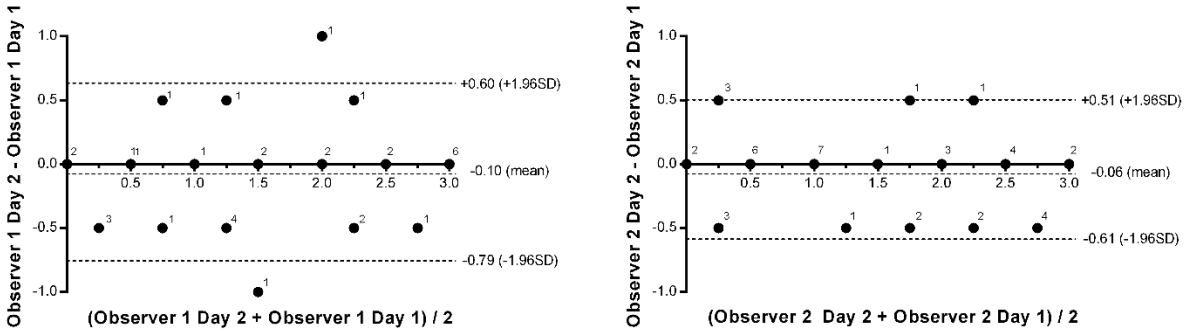


Figure 4-9: Left: Limit of agreement plot showing intra-observer repeatability of observer 1 using the new 7 point scale. Right: limit of agreement plot showing intra-observer repeatability of observer 2 using the new 7 point scale. Numbers at each point indicate the number of overlapping points.

4.4.6 Superior and inferior eyelid repeatability for the new 7 point scale.

The mean difference, with 95% limits of agreement, and CCC for the superior eyelid was -0.02 (-0.60 to 0.55), CCC=0.94. For the inferior eyelid, the mean difference, 95% limits of agreement were 0.14 (-0.41 to 0.69), with CCC=0.96. The repeatability for the superior and inferior eyelids were approximately the same. Figure 4-10 compares the repeatability of grading superior and inferior eyelids in the K5M with the new 7 point scale.

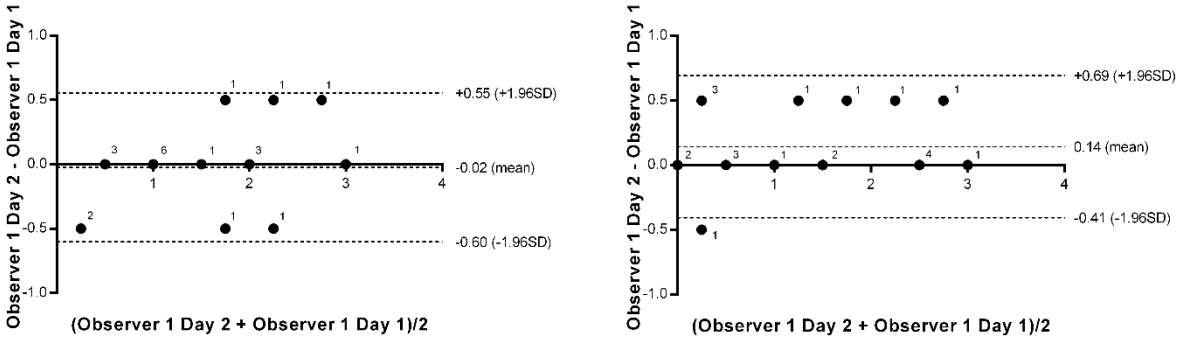


Figure 4-10: Left, limit of agreement plot showing intra-observer repeatability of observer 1 using the new 7 point scale to grade the superior eyelid with the K5M. Right: limit of agreement plot showing intra-observer repeatability of observer 1 using the new 7 point scale to grade the inferior eyelid with the K5M. Numbers at each point indicate the number of overlapping points.

4.4.7 Semi-objective digital grading

Semi-objective digital image analysis (Figure 4-11) was conducted by a single observer on the images from the first grading experiment. The mean percentage dropout (mean ± SD) in K4 and K5M images were 27.5% ± 17.6% and 29.5% ± 16.7% respectively.

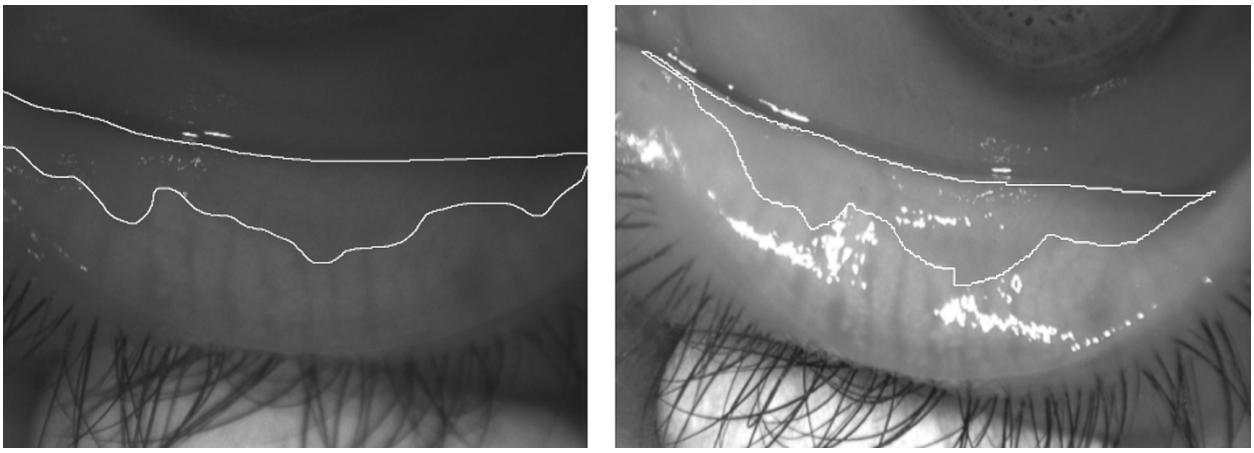


Figure 4-11: The free-hand tool in ImageJ was used to quantify gland dropout in K4 (left) and K5M (right) images. The K5M images appeared brighter, with better contrast and larger field of view than the K4 images.

Despite a statistically significant correlation between the measured dropout between the K4 and K5M (Pearson's $r = 0.80$, $p < 0.001$), a method comparison plot reveals the inconsistencies and variability in measured percentage dropout of the K4 and K5M images. The coefficient of repeatability is 21.8%, and the 95% limit of agreement interval spanned from -19.8% to +23.8 (Figure 4-12).

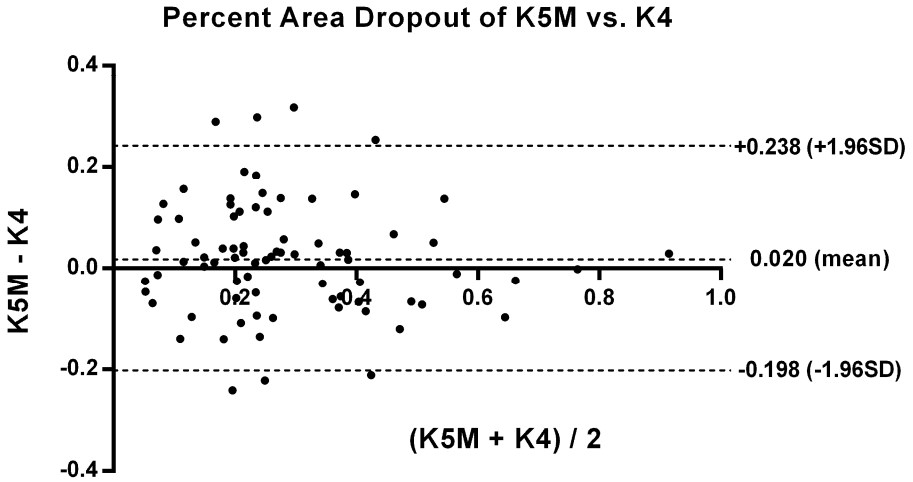


Figure 4-12: A method comparison plot showing variability between percent area dropout measured on K4 versus K5M images. A scale unit of 0.2 corresponds to 20%.

4.5 Discussion

Both the inter- and intra-observer mean difference was <<1 grade points for the K4, K5M and both K4 and K5M together (all ≤ 0.18; Table 4-4), which could be rounded to 0 since the meibography grading scale consisted of only integers. This indicated that the inter- and intra-observer mean difference for the two observers was negligible when using this Arita 4 point integer scale, and that the responses from the two observers were similar (Figure 4-3).

The standard deviation of the mean differences was approximately ±0.50 for both inter- and intra-observer differences, resulting in a coefficient of repeatability of approximately:

$$(1.96)(0.50) = 0.98$$

If μ represents the inter-(or intra-)observer mean difference, then a 95% confidence interval for limits of agreement can be expressed as:

$$[\mu - 0.98, \mu + 0.98]$$

Since $\mu \approx 0$ as discussed before, this indicates that on this Arita 4 point meibography scale, 95% of the time both observers will grade within -1 to +1 grade units of each other and against themselves.

Concordance correlation coefficient is a measure of repeatability and can be used to quantify agreement of observers and devices.[26] The CCC of inter-observer repeatability of the K4 was very similar to the K5M, but the K5M showed a slightly higher intra-observer CCC than the K4. Despite the higher intra-observer CCC of the K5M over the K4 (Table 4-4), this was not clinically significant, as the observers still graded within -1 to +1 grades against themselves, 95% of the time (Figure 4-4).

The limits of agreement spanning -1 to +1 covered 3 units of the Arita 4 point scale, which made both grades 1 and 2 very convenient choices if the observer was in doubt. Considering that the eyelids have approximately 26 glands each and that 1 unit of progression is 1/3 of total area MG loss, it would require 8 – 9 glands to drop out before the unit of progression can be increased. This suggests that the Arita 4 point

scale is insensitive to MG dropout progression. Bailey et al.[28] proposed that a clinical grading scale of moderate sensitivity should have a paired comparison concordance of no more than 37%, meaning that two observers should not agree more than 37% of the time, and no more than 13% for a scale of fine sensitivity. In this study, it was found that the two observers agreed 69-76% of the time with each other, and against themselves which indicated that the sensitivity of this Arita 4 point meibography scale could be improved. It is also important to note that a majority of participants in this study had either grade 0 or 1 level dropout, which caused the grading distribution to take on a binary (and coarser) characteristic. With the digital imaging capabilities of the K4 and K5M, it should not be unreasonable for the observers to detect changes smaller than 1/3 total area dropout. It was unlikely that the observers would fail to discriminate between dropout of, for example, 7 MGs versus 2 MGs. Therefore, to maximize the potential of digital imaging, a meibography scale of finer sensitivity (e.g. incorporate 0.5 unit steps) was considered.

When the second repeatability experiment was conducted with a finer scale (7 point scale), the maximum rate of agreement had reduced slightly from 76% to 64%. Although the 95% limits of agreement intervals had narrowed, the inter-observer repeatability was still within 0.5 to 1.0 grades of each other (Figure 4-8). It is also important to note that the CCC values had also increased (from ~0.78 to ~0.92) using this new scale, indicating an improvement over the Arita 4 point scale. The 95% limits of agreement intervals for intra-observer repeatability may be small enough that observers could grade within 0.5 points against themselves (e.g. intra-observer repeatability of observer 2, Figure 4-9). While this suggests that the 7 point scale may have some utility, this grading method is still not a very sensitive method. A precision of ±0.5 grade points would still not be able to discriminate a dropout of, for example, 2 whole MGs, but will still allow better discrimination of severity of MG dropout than the Arita 4 point scale.

While the inter- and intra-observer repeatability of each device had been established, using ImageJ to measure percentage area dropout revealed that there was a considerable lack of agreement between the K4 and K5M images (Figure 4-12). As a result, although both devices are suitable as stand-alone device for MG imaging, we recommend that patients be followed up on any one device only, and that clinicians should

not interchange devices to monitor MG dropout. While digital analysis might have the potential to be more discriminatory, grading scales are more likely to be used in clinical practice.

A possible factor that contributed to the variable nature of the data may be difference in quality of image. The K5M had been optimized for meibography with optimal IR diode positioning, increased working distance and enhanced image views. This allows the K5M to generate images that have improved exposure and are clearer to observe. In contrast, the IR illumination system in the K4 was not intended for meibography, but instead for pupillometry. As a result, gland borders on the K4 appear fuzzy, and appear to blend into the background and are not easily distinguishable (Figures 4-1 & 4-11). There is also uncertainty in defining the borders of the tarsal plate, which is compounded by the fact that the K4 has a more restricted field of view. These uncertainties make it difficult to select areas of interest to calculate dropout.

It is also important to note that the ImageJ percentage dropout is derived from selecting areas where glands are absent and is not a real measure of MG dropout. To measure actual gland shortening and dropout the position of pre-atrophied MGs would have had to be measured, but since this is not a longitudinal-type study, atrophic changes cannot be quantified in this case. Also, atrophied gland structures are not detected by IR light, and therefore it is impossible to determine the amount atrophied by the time imaging was performed.

Although the method of eyelid eversion was kept consistent for each participant as they were being imaged across each device, it was not possible to perfectly evert an eyelid to expose identical areas each time. This resulted in inconsistency in exposing glands, which may have contributed to variability to the data. A direct consequence of this was the consistently better repeatability in superior eyelid grading compared to the inferior eyelid. The eversion of the superior eyelid was more consistent possibly because the eyelashes of the everted eyelid could be pinned to the orbital bone. In the inferior eyelid, the eversion is facilitated by a "rolling" motion that could expose a variable amount of palpebral conjunctiva each time. Furthermore, the larger surface of the superior eyelid surface allowed the observer to see where the termination of the glands

were, and allowed for easier interpretation of dropout. In the inferior eyelid, there were occasions encountered during this study where the termination of the glands could not be observed despite the best efforts to evert them. This may explain why grading the superior eyelid was more repeatable than the inferior eyelid.

Therefore, everting the participants' eyelids once, and then imaging them 3 times consecutively may result in lower variability, but repositioning the participant's head in-between imaging was an attempt to mimic real-world practice. For example, a patient imaged on one occasion may return in a month to be imaged again, and the positioning of their head and eyelid eversion will be slightly different each time. Therefore it was decided to have the patient retract and reposition their head in the instrument for every subsequent image.

4.6 Conclusion

Although there is a difference in image quality between the K4 and K5M, inter- and intra-observer repeatability was found to be very similar for each device separately. When using the instruments to take meibography images and grading them with the linear Arita 4 point integer scale, it can be expected that two observers will grade within -1 to +1 grade of each other and within themselves. Due to differences in image quality and properties between the two devices, it is recommended that patients be imaged on only one device. To take advantage of the benefits of digital imaging, a grading method with higher sensitivity (e.g. a 7 point scale) should be used with these two instruments.

5 A Comparison of Dry Eye Diagnostic Tests between Symptomatic and Asymptomatic Age-Matched Females

5.1 Overview

PURPOSE: To determine the strength of association of dry eye (DE) diagnostic tests to DE symptoms in an age-matched female cohort.

METHODS: Twenty females symptomatic of DE (Ocular Surface Disease Index, OSDI, ≥ 13) were age-matched with 20 asymptomatic females (OSDI < 13) in this cross-sectional study. Non-invasive tear breakup time (NIBUT), ocular staining, meibum quality, number of glands obstructed, lid wiper epitheliopathy (LWE), Marx's line placement, eyelid margin score, Schirmer's test, meibography, and visual acuity were compared between the two groups.

RESULTS: The symptomatic group showed significantly poorer NIBUT, meibum quality, number of obstructed glands, and ocular staining than the asymptomatic group. The diagnostic tests (area under curve, odds ratio) most strongly associated with DE symptoms were ocular staining (93, 5.0), number of glands obstructed (79, 2.6), meibum quality (76, 2.4), and NIBUT (74, 3.2). Marx's line placement, LWE, eyelid margin score, Schirmer's test, meibography, and visual acuity were not significantly different between the two groups ($p > 0.05$) and were not significantly associated with DE symptoms.

CONCLUSION: The diagnostic tests most strongly associated with symptoms in older women were ocular staining, meibum quality, number of glands obstructed, and tear film stability.

5.2 Introduction

Dry eye (DE) disease is a prevalent condition affecting millions around the world.[1-5] The disease can be broadly categorized based on two different etiologies – due to lack of aqueous tear production (aqueous deficient DE), or due to increased tear film evaporation (evaporative DE).[6] Some of the risk factors associated with DE include age,[7,8] female sex,[9] asian ethnicity,[10] use of various medications,[11-13] and certain health conditions (e.g. rheumatoid arthritis[14] and Sjögren's Syndrome[15]).

Dry eye causes symptoms of discomfort that are typically described as burning, stinging, tearing and or itching.[16] These symptoms, if severe enough can become bothersome and can be destructive towards quality of life and work productivity.[17] In the United States, the economic burden of DE is estimated to cost $55.4 billion to society, with an average DE patient spending approximately $783 yearly for DE management alone.[18]

The definition of DE disease was revised several times, with the most recent definition by the 2007 International Dry Eye Workshop to be "…a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface."[6] This definition highlights the vague and elusive nature of the disease.

The assessment of DE disease is conducted using a wide variety of clinical tests.[16] Ocular staining,[19,20] tear film stability,[21] Schimer's test,[22] eyelid margin examination,[23] and meibomian gland (MG) function[24] are tests that are commonly used to assess the severity of DE disease. These tests are usually combined with a subjective symptom measurement (e.g. Ocular Surface Disease Index (OSDI),[25] Symptom Assessment iN Dry Eye (SANDE),[26] Standard Patient Evaluation of Eye Dryness (SPEED))[27] to obtain information on patient morbidity and quality of life. Monitoring changes in these test results over time provides information about how the disease or therapy is progressing.

Recently, Marx's line placement[28-30] and lid wiper epitheliopathy (LWE)[31-34] were found to play a role in DE disease.[35] Marx's line is a junction where the keratinized epithelium of the eyelids meets the epithelium of the conjunctiva.[36] This junction is located at the eyelid margin, posterior to the MG orifices and pinned at the leading edge of the tear meniscus.[29] The anterior migration of the Marx's line was thought to be due to cellular changes of the epithelium.[28] The cause for this was not entirely known, however the anterior migration of Marx's line appeared to be associated with meibomian gland dysfunction (MGD).[37] The mechanical debridement of Marx's line was found to improve signs and symptoms of DE.[35,38]

The eyelid wiper is an epithelial ridge located posterior to the MGs, underneath the superior eyelid. This area is characterized by a combination of stratified epithelium, cuboidal cells, goblet cells and parakeratinized cells.[39] The lack of lubrication and increased mechanical friction at the lid wiper and corneal interface was thought to cause the epithelia of the eyelid wiper to undergo cellular changes.[39] These changes could be readily observed with topical ophthalmic dyes.[32] A recent study found a higher prevalence of LWE in DE participants,[32] and another study found that LWE is correlated with DE symptoms.[40]

Since DE disease is associated with both age[7,8] and sex,[9] it is not clear whether or not the presence of LWE and a thickened Marx's line is due to DE disease, or as a result of confounding factors from older age and female sex. No previous studies that examined LWE and Marx's line placement have used an age-matched case-control study design. The purpose of this study was to firstly, compare LWE and Marx's line placement in an age-matched design between symptomatic and asymptomatic females. Secondly, this study also aimed to place LWE and Marx's line placement in the context of other diagnostic tests for DE disease.

5.3 Methods

The study was conducted at the Centre for Contact Lens Research (CCLR), at the University of Waterloo (UW). The study was conducted in conformance with the ethical principles of the Declaration of Helsinki, the ICH guidelines for Good Clinical Practice, the UW Guidelines for Research with Human Participants.

Informed consent was obtained from all participants prior to enrollment in the study. Ethics clearance was obtained through a UW Research Ethics Committee prior to commencement of the study.

5.3.1 Participants

This was a cross-sectional, age-matched study that enrolled 40 females. Twenty females with OSDI score ≥ 13 were enrolled, and were age-matched (±3 years) with asymptomatic females (OSDI < 13). All participants who were on ocular lubricants were asked to abstain from using them on the day prior to the visit appointment, and participants taking systemic medications were required to have used the same dosage and regimen for at least 3 months. The inclusion and exclusion criteria for study entry is outlined in Table 5-1.

Table 5-1: Criteria for study entry.

Inclusion	**Exclusion**
Participants must have read an information consent letter.	Has any active ocular disease, infection, or allergies.
Must be at least 17 years of age and has full legal capacity to volunteer.	Has a systemic condition or on systemic medications that may affect an outcome variable.
For the symptomatic group, participants must have OSDI ≥ 13 and be female.	Has undergone refractive error surgery.
For the asymptomatic group, participants must have OSDI < 13, and can be age-matched (±3 years) to a participant in the symptomatic group.	Has worn contact lenses within the past 5 years.
Participants must be willing and able to follow instructions to maintain the appointment schedule.	Has known sensitivity to diagnostic pharmaceuticals to be used in the study.
	Is pregnant, lactating, or planning a pregnancy at the time of enrolment.
	Is participating in any concurrent clinical or research study.

5.3.2 Clinical Methods

All clinical tests were conducted by one investigator, in the order they appear below. The clinical grading scales for meibum quality (Table A1),[41] eyelid margin score (Table A2), LWE (Table A3),[34] Marx's line placement (Table A4),[42] and MG dropout (Table A5)[43] are listed in the Appendix.

At the beginning of each visit, demographic information was collected and was followed by a symptom assessment using the OSDI.[25] The OSDI is a validated questionnaire that queried 3 aspects of DE: vision-related aspects, ocular symptoms, and environmental triggers.[25] Based on a previous study that investigated clinically minimal differences,[44] participants who scored higher than 12 were considered to be symptomatic.

Visual acuity was assessed using a computerized high contrast chart at a distance of 6 meters. Participants were instructed to read rows consisting of 5 letters that progressively decreased in size until they could no longer distinguish the letters on the screen. Visual acuity was recorded in logMAR form.

Non-invasive tear breakup time (NIBUT) was assessed by projecting illuminated Placido disks onto the anterior tear film.[45] Participants were asked to blink several times before holding their eyes open for as long as they could. The time in seconds from eye opening to the first disruption of the Placido rings to occur was recorded. Three measurements were taken from each eye and averaged.

Meibum quality was assessed by applying firm digital pressure to the inferior eyelid margin for approximately 8 seconds to express the central 8 MGs. The quality of expressed meibum was graded using the scale described in Mathers et al.[41] (Table A1). The number of glands that did not express meibum when digital pressure was applied was counted.

Ocular staining was assessed with a fluorescein strip (Fluorets, Bausch & Lomb Canada Inc., Markham, Ontario, Canada) wetted with saline (Bausch & Lomb Sensitive Eyes® Saline Plus, Rochester, New York, USA). With the excess shaken off, the moistened strip was touched to the inferior fornices of both eyes. The van Bijsterveld grading scale[46] was used to grade the extent of ocular surface staining (both corneal and conjunctival) and the full scale from 0-9 was utilized. Fluorescein was used in place of Rose Bengal as it was not permitted for use in Canada. Despite this, there was no difficulty in assessing conjunctival staining.

The eyelid margin was examined under slit lamp biomicroscopy. The degree of erythema, lash loss, edema, and lid margin telangiectasia were each graded separately. The eyelid margin score was derived from the sum of the grades of all these features.

Lid wiper epitheliopathy, as well as Marx's line placement was assessed as follows. Fluorescein was instilled twice, one minute apart, in both eyes. After an additional 3 minutes had elapsed, the superior eyelids of both eyes were everted and fluorescein LWE was graded. A Lissamine green strip (GreenGlo™, HUB Pharmaceuticals, Rancho Cucamonga, California, USA) was wetted with saline, and with the excess shaken off, instilled in the inferior fornices of both eyes. The inferior eyelids were everted to grade Marx's line placement using the scale described in Kim et al.[42]

After a minute had elapsed from the previous Lissamine green instillation, another Lissamine green strip was instilled. Then, after 3 minutes had elapsed, the superior eyelids of both eyes were everted to grade Lissamine green LWE staining. The final LWE grade was the average of the fluorescein and the Lissamine green LWE grade.

Meibomian gland atrophy was assessed using the Keratograph 5M (OCULUS, Wetzlar, Germany).[47] The superior and inferior eyelids were everted and the meibo-scan tool was used to capture images of the MGs. Meibomian gland dropout was graded with the Arita scale. [43] The score for both the superior and inferior eyelid were summed to generate the "meiboscore" for each eye.

Schirmer's test was conducted by inserting a Schirmer's tear strip (TearFlo™, HUB Pharmaceuticals, Rancho Cucamonga, California, USA) in both eyes at the junction between the temporal third and central third of the eyelid margin.[48] Participants were instructed to close their eyes during the test. The tear strips were removed after 5 minutes and the length of wetting was measured. No anesthesia was used.

5.3.3 Statistical Analysis

Data from only the right eye were analyzed. Statistical analyses were conducted using GraphPad Prism 6.05 (GraphPad Software, California, USA). Normal data distribution testing was conducted using the Shapiro-Wilk test at the level of $\alpha=0.05$.

Paired t-test or Wilcoxon signed-rank test (depending on the data distribution) was used to determine the significance of the difference of means of the diagnostic tests between the two groups. Similarly, Pearson's or Spearman's rho was used to determine the linear correlation between diagnostic tests. Receiver operator characteristics (ROC) were used to determine sensitivity and specificity of the diagnostic tests, and their corresponding cut-offs for classifying symptomatic and asymptomatic participants. Level of statistical significance was set at $p < 0.05$.

5.4 Results

5.4.1 Participants

A total of 40 women (20 matched pairs) completed the study. In the symptomatic group, participants reported being symptomatic for DE for at the past 3 years. Six of the 20 women in the symptomatic group verbally expressed that they had Sjögren's Syndrome, but this was not medically confirmed at the visit appointment. The median age (interquartile range, IQR) of the symptomatic group was 60 (15) and asymptomatic group was 62 (15). The age range of this sample was 46 to 73 years old inclusive.

5.4.2 Clinical Outcomes

The clinical results are summarized in Table 5-2. The symptomatic group showed significantly higher ocular staining (+5.0 grade points), lower NIBUT (-0.9s), poorer meibum quality (+1.0 grade points), and had a higher number of obstructed MGs (+2.0 glands) than the asymptomatic group (all $p \leq 0.01$ by Wilcoxon signed-rank test). Furthermore, the symptomatic group also showed greater amount of MG atrophy (+0.5 grades) and a more anteriorly placed Marx's line (+1.0 grades), however these differences

were not statistically significant (all $p > 0.06$ by Wilcoxon signed-rank test). Eyelid margin score and LWE were similar in both groups (both $p > 0.57$ by Wilcoxon signed-rank test). Schirmer's test was higher in the symptomatic group but this difference was not statistically significant ($p = 0.87$ by Wilcoxon signed-rank test).

The linear correlations between all DE diagnostic tests are displayed in Table 5-3. Symptoms did not significantly correlate with any diagnostic tests (all $p>0.05$). Marx's line placement correlated significantly with eyelid margin score and ocular staining, but lid wiper epitheliopathy did not significantly correlate with other diagnostic tests.

The ROC values of diagnostic tests are shown in Table 5-4. The diagnostic tests that showed a significant association ($p < 0.05$) to symptoms were ocular staining (odds ratio, OR: 5.0), number of glands obstructed (OR: 2.6), meibum quality (OR: 2.4) and NIBUT (OR: 3.2), with area under curve (AUCs) of 93, 79, 75, and 74 respectively. Meibography and Marx's line placement were similar in AUCs (64) but was not significantly associated with symptoms. Lastly, LWE, eyelid margin score, Schirmer's test, and visual acuity were all weakly associated with symptoms (Table 5-4).

Table 5-2: Summary of clinical findings (first quartile, median, third quartile). All comparisons were conducted using the Wilcoxon signed-rank test.

	Symptomatic Group	Asymptomatic Group	p value
Age	51, 60, 66	52, 62, 67	0.43
OSDI (0-100)	21.3, 35.4, 56.7	0.0, 3.1, 6.7	**<0.01**
Visual Acuity (logMAR)	-0.08, 0.04, 0.10	-0.08, 0.02, 0.12	0.86
Ocular staining (0-12)	3.2, 5.5, 7.0	0.0, 0.5, 1.0	**<0.01**
Meibum quality (0-3)	3.0, 3.0, 3.0	2.0, 2.0, 3.0	**<0.01**
Number of glands obstructed (0-8)	6.0, 7.0, 8.0	2.0, 5.0, 6.8	**<0.01**
NIBUT (seconds)	1.9, 2.1, 2.6	2.3, 3.0, 5.3	**0.01**
Eyelid margin score (0-13)	4.2, 7.0, 8.0	6.0, 8.0, 9.0	0.58
Meiboscore (0-6)	1.0, 2.0, 3.0	1.0, 2.0, 2.0	0.09
Lid wiper epitheliopathy (0-3)	0.0, 0.0, 1.0	0.0, 0.0, 0.4	0.71
Marx's line placement (0-2)	0.0, 1.0, 2.0	0.0, 0.0, 1.0	0.06
Schirmer's Test (0-30)	3.5, 11.0, 15.8	3.2, 8.5, 14.0	0.87

Bold represents $p < 0.05$

Table 5-3: Correlation matrix showing the linear relationship between diagnostic tests with Spearman's rho correlation coefficient.

	OSDI score	Meibum quality	Number of glands obstructed	Meibography	NIBUT	Eyelid margin score	Ocular Staining	Visual Acuity	Schirmer's test	Marx's line	Lid wiper
OSDI score		-0.12	-0.10	0.30	-0.18	0.09	0.34	0.33	-0.11	0.24	-0.28
Meibum quality			**0.57**	**-0.47**	-0.42	0.25	0.39	0.32	0.10	0.25	0.33
Number of glands obstructed				-0.06	-0.33	0.18	0.17	0.29	0.31	0.13	0.16
Meiboscore					-0.25	0.11	0.15	0.12	-0.32	0.01	-0.32
NIBUT						**-0.46**	**-0.68**	**-0.60**	0.27	-0.31	-0.43
Eyelid margin score							**0.58**	0.34	-0.27	**0.60**	0.16
Ocular Staining								**0.46**	**-0.56**	**0.58**	0.29
Visual Acuity									-0.05	0.06	0.04
Schirmer's test										-0.36	-0.12
Marx's line											0.04
Lid wiper											

Bold represents $p < 0.05$

Table 5-4: Area under ROC curves for diagnostic tests.

Diagnostic Test	Area Under Curve	Sensitivity	Specificity	Cutoff	Odds Ratio
Ocular staining (0-12)	**0.93**	100	80	>1.5	5.0
Number of glands obstructed (0-8)	**0.79**	90	65	>5.5	2.6
Meibum quality (0-3)	**0.76**	85	65	>2.5	2.4
NIBUT (seconds)	**0.74**	80	75	<2.6	3.2
Meiboscore (0-6)	0.64	47	84	>2.5	3.0
Marx's line placement (0-2)	0.64	30	90	>1.5	3.0
Eyelid margin score (0-13)	0.56	80	40	<8.5	1.3
Lid wiper epitheliopathy (0-3)	0.54	40	70	>0.12	1.3
Schirmer's test (0-30)	0.53	40	80	>14.5	2.0
Visual acuity (logMAR)	0.51	90	25	<0.11	1.2

Bold represents $p < 0.05$

5.5 Discussion

By controlling for age and sex, this study allowed for the examination of DE diagnostic tests in the absence of two well-known confounding factors. Despite having controlled for age and sex, this study did not find a linear correlation between symptoms and signs and reinforced the findings of previous studies.[49,50] However, based on odds ratios and AUCs, the tests that were relatively strongly associated with symptoms were ocular staining, number of central obstructed glands, meibum quality, and NIBUT. In contrast, eyelid margin score, LWE, Schirmer's test, and visual acuity each had very low AUCs and did not appear to be

associated with symptoms. Overall, the symptomatic group exhibited worse physical features of DE disease than the asymptomatic group.

The placement of Marx's line was found to be more shifted anteriorly in the symptomatic group than the asymptomatic group, but this difference was not significant. A previous study[37] had found an association between an anteriorly shifted Marx's line with MGD, however the grading scale used in that study was much more sensitive than the one used in this present study. Yamaguchi assigned a score to the Marx's line at each third of the eyelid and summed them, yielding a total score out of 9. The grading scale used in this study had only 3 grades and each of these grade units also represented more than one clinical feature. This made the current scale less sensitive and less discriminative and may explain why a difference between the two groups was not detected.

One of the major differences between the two groups was the quality of meibum, with the symptomatic group showing significantly poorer meibum quality than the asymptomatic group. Increased viscosity of meibum is partly responsible for driving MG obstruction.[51] The obstruction of MGs would then cause the tear film lipid layer to become deficient, leading to the rapid collapse and destabilization of the tear film onto the ocular surface.[52] With decreased tear film stability, the ocular surface is repeatedly exposed and desiccated, resulting in constant stimulation of the ocular surface and driving discomfort symptoms.[53] The repeated desiccation of the ocular surface also results in the increased uptake of ophthalmic vital stains.[54] This mechanism may be an over-simplification of the disease pathophysiology, but treatment in DE that aimed to decongest the MGs and improve meibum quality have resulted in substantial improvements in DE symptoms and signs.[55-57]

The meibum quality and the degree of gland obstruction were unusually poor in this sample. In this relatively older age group this could be plausible as changes in meibum quality were found to be related to aging.[58] The poor NIBUT in both groups may have been a direct consequence of this. There may be other etiological factors that have contributed to the severity of MGD, but this was not investigated in this study.

Since the function of MGs was to produce meibum, the atrophy of the MGs would therefore result in decreased production of meibum. This study did not find a correlation between gland dropout and number of obstructed glands. Furthermore, this study unexpectedly found an inverse relationship between meibum quality and gland dropout, suggesting that participants with worse MG atrophy were producing relatively healthier lipids. This finding is contradictory to the findings from another study.[59]

Only one investigator conducted all the clinical tests in this study. While this eliminated the possibility of inter-observer variabilities, this also meant that it was impossible to mask participant grouping. Consequently, the results may show some bias. Additionally, the enrollment of participants were based only on symptoms and not on any of the diagnostic tests for DE. This was necessary to evaluate how well diagnostic tests discriminate symptomatic from asymptomatic participants. If certain diagnostic values were required for entry, then the ROC analyses would show an artificially high ability for those tests to distinguish the two groups.

Since this study observed a sample of women in the age range between 46 and 73, the results of the study may not be valid for younger women or males. However, the findings are still valuable, as the elderly population is expected to rise globally over the next few decades,[60] and consequently the prevalence of DE as well.[7,8] In addition, post-menopausal women form a substantial proportion of the sufferers of DE.[61] For future work, it would be valuable to conduct a similar study in a different age group, in males, or even with contact lens wearers. By examining the strength of associations of diagnostic tests with symptoms, it may provide some information about the underlying pathophysiology.

5.6 Conclusion

The DE tests that were strongly associated with DE symptoms in older women in this study were ocular surface staining, meibum quality, number of obstructed glands, and NIBUT.

6 The Effect of an Eyelid Warming Device on Meibomian Gland Dysfunction

6.1 Overview

PURPOSE: To determine the effectiveness of the MGDRx® EyeBag in the management of meibomian gland dysfunction.

METHODS: This was a prospective, randomized, controlled, single-masked, bilateral eye study that enrolled 29 participants. Participants were randomized into either the EyeBag group or the control group. Participants in the EyeBag group were required to use the EyeBag 10 minutes 2x/day, and the control group remained on their own dry eye treatment regimen (if applicable). All participants were seen at baseline, 2 weeks and 4 weeks. At 4 weeks, participants in the EyeBag group were asked to stop using the EyeBag. All participants were seen again at the 8 week mark. Primary outcomes were the Ocular Surface Disease Index (OSDI), Current Symptoms Questionnaire (CSQ), meibomian gland score (MG score), and non-invasive tear breakup time (NIBUT).

RESULTS: Twenty five participants completed the study (mean age 38±15 years, 7 male). There was a significant change in OSDI over time for the EyeBag group (mean values, baseline: 39.1, week 2: 26.8, week 4: 26.6, week 8: 27.7; $p<0.05$), but no significant change in the control group. There was a significant improvement in symptoms immediately after conducting the EyeBag based on at-home CSQ scores (Δ=-5.0 points, $p<0.05$), but no significant change in the control group. There was no significant change in MG score and NIBUT over time for either group.

CONCLUSIONS: The MGDRx® EyeBag was effective at relieving symptoms in participants with dry eye, but the effect on meibomian gland function and tear stability when used for only 4 weeks was modest.

6.2 Introduction

Meibomian glands (MGs) are modified sebaceous glands imbedded within the tarsal plate.[1] The glands consist of multiple acini which manufacture and secrete meibum (oils) into a central duct. The duct terminates in an orifice at the eyelid margin posterior to Marx's line. Meibum released at the orifice serves to mix and stabilize the tear film.[2]

When the gland orifices becomes obstructed, the delivery of meibum onto the tear film is reduced.[1] The primary mechanism of gland orifice obstruction appears to be driven by hyperkeratinization and thickening of the MG ductule epithelium.[3,4] This results in a loss of tear film stability[2] and brings about symptoms of dry eye (burning, stinging, foreign body sensation).[5] Symptoms of dry eye range from mild to severe[6] and can potentially have a large impact on economic burden[7] and quality of life of the patient.[8] This condition is termed meibomian gland dysfunction (MGD).[6] As per the 2011 International Workshop on Meibomian Gland Dysfunction, the recommended definition of MGD is:

> "… a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. This may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease."[9]

Meibomian gland dysfunction is thought to be the leading cause of dry eye.[6] The prevalence of MGD ranges widely and have been reported to be from 3.5[10] to 69.3%,[11] depending on ethnicity and the working definition of MGD used for study inclusion.[12] The condition appears to be associated with, but not limited to, aging,[13,14] taking certain medications (e.g. retinoids),[15-18] androgen deficiency,[19-21] and possibly contact lens wear.[12,22]

One of the mainstay treatments for MGD involves warm compresses.[23] The application of heat and pressure to the eyelid margin melts the meibum plugging the gland orifices and facilitates delivery of meibum from the gland into the tear film. One standard recommendation is to wet a face towel with hot water and apply

the towel to the eyelid margins.[24] This procedure has limitations, such as the inconvenience of constantly holding the towel against the face, rapid heat loss from the towel, and the constant need to replace and reheat the towel.[25,26]

Recently, a number of commercial products were developed with the same principle of delivering either moist or dry heat to the eyelids and all serve to retain heat better than a face towel.[25,27-30] The MGDRx® EyeBag (The EyeBag Company Ltd, Halifax, UK) is one such product[31] and is essentially a cotton/silk pouch filled with flax and linseed.[32] The product retains heat well and can be easily fitted over the closed eyes without difficulty.[25]

At the time of conducting this study, a PubMed search using the terms "mgd" and "eyebag" revealed only one other study examining the treatment effect of the EyeBag on MGD.[28] The previous study examined the effectiveness of the EyeBag using a contralateral eye design and found a large improvement in symptoms and modest improvement in MG function in the eye receiving treatment. The purpose of this current study is similar, in that it examines the effectiveness of the EyeBag in improving MG function and symptoms, however the main difference is in the study design (bilateral) which we feel allows for more accurate representation of symptom change.

6.3 Methods

6.3.1 Participants

This study was conducted in conformance with the ethical principles of the Declaration of Helsinki, the ICH guidelines for Good Clinical Practice, and the UW Guidelines for Research with Human Participants. Informed consent was obtained from all participants prior to enrollment in the study. Ethics clearance was obtained through a UW Research Ethics Committee prior to commencement of the study.

This was a prospective, randomized, controlled, single-masked, bilateral eye study that enrolled 29 participants.

The sample size was determined using G*Power 3.1 (Universität Kiel, Kiel, Germany).[33,34] To detect a 3.0 mean difference in MG score (1.0 effect size) at 90% power at 0.05 level of significance, a minimum sample size of 24 is required. The 3.0 mean difference was chosen since it was a modest value compared to the reported 7.0 mean difference previously reported with another device that has been developed for the management of MGD, the TearScience® LipiFlow.[35]

The inclusion and exclusion criteria are outlined in Table 6-1.

Table 6-1: Criteria for study entry

Inclusion	**Exclusion**
Has read and signed an information consent letter.	Is currently participating in any concurrent clinical or research study.
At least 17 years of age.	Has a systemic condition or on medication that may affect an outcome variable (e.g. previous refractive and/or cataract surgery, glaucoma medications, Sjögren's syndrome, rheumatic disease).
Ocular Surface Disease Index ≥ 23.	Hypersensitivity to EyeBag components.
Meibomian Gland Score ≤ 9 (see below in Methods).	Is pregnant, lactating or planning a pregnancy at the time of enrolment, as determined verbally.
Not a contact lens wearer.	Has undergone refractive error surgery.
Is on a stable dry eye regimen (if applicable) within the past 4 weeks, and is willing to maintain this regimen.	Has significant discomfort and or inducible seizures from rapid blinking lights.
Agree to use the MetricWire app for online data entry.	

6.3.2 Visit Schedule

This study consisted of 4 visits. At the initial visit, participant eligibility was determined and baseline data were collected. Participants were randomized into either the EyeBag (treatment) or control group. Participants in the control group were asked to remain on their current dry eye regimen and keep medications steady for the duration of the study period. Participants in the EyeBag group were provided the EyeBag and instructed to use them 10 minutes twice daily. All participants returned at 2 weeks and 4

weeks for follow-up assessments. After 4 weeks, participants in the EyeBag group ceased EyeBag use and all participants returned for a final assessment at the 8 week mark.

6.3.3 Clinical Methods

At the beginning of each follow-up study visit, a research assistant asked participants how often they had used the EyeBag at home. Participant compliance information regarding frequency and duration of use was documented. Changes to health or medications were also documented. All clinical tests were conducted by one investigator (WN) and in the same order each time as they appear below.

Dry eye symptoms were measured using the Ocular Surface Disease Index (OSDI)[36] and the Symptom Assessment iN Dry Eye (SANDE)[37] questionnaires. The OSDI is a questionnaire that assessed severity of dry eye by querying symptoms, difficulties with certain tasks, and comfort in different environments.[36] The SANDE consisted of visual analogue scales that queried severity and frequency of dry eye symptoms.[37]

Tear film lipid layer thickness was conducted using the LipiView (TearScience®, Morrisville, North Carolina, USA) in primary gaze.[38]

High and low contrast visual acuity was assessed using an electronic computer LogMAR chart at an optical length of 6 meters. Participants were asked to read rows of letters that decreased in size until no more letters could be read.

Non-invasive tear breakup time was assessed by using the Humphrey Atlas® Topographer 991 (Zeiss, California, USA). The topographer projected illuminated Placido discs onto the cornea[39] and the time for distortion to appear on the Placido disks during the interblink interval was recorded 3 times and averaged.

Corneal staining and conjunctival staining was assessed by wetting a fluorescein strip with a few drops of sterile saline, shaking off the excess, and instilling it in both eyes. Corneal and conjunctival staining was assessed using the CCLR scale (corneal: 0 to 100 for type, extent, and depth; conjunctival: 0 to 100),[40] both with cobalt blue illumination through a Wratten no. 12 barrier filter.

Meibomian gland assessment was conducted using the Meibomian Gland Evaluator (TearScience®, Morrisville, North Carolina, USA).[41] The leading edge was applied against the central inferior eyelid, exerting a pressure of 1.25g/mm^2. The Meibomian Gland Score (MG score) is the grade of the appearance of meibum expressed from the 5 central glands:[35]

Grade 0: No secretion

Grade 1: Inspissated

Grade 2: Coloured liquid

Grade 3: Clear oil

The Meibomian Glands Yielding Liquid Secretions (MGYLS) variable is a count of the number of glands with MG score 2 or higher.

Meibography was assessed using the meibography feature on the Keratograph® 5M (OCULUS, Wetzlar, Germany).[42] Both the inferior and superior eyelids of both eyes were everted and imaged. Gland dropout was graded using the Arita scale[43] and the final score for each eye was obtained by summing the grade from the upper and the lower eyelids.

During the study visit, participants in the EyeBag group received an EyeBag treatment. The EyeBag was heated in a 900W microwave (RCA, New York, USA) on high power for 30 seconds and then placed over the participants' closed eyes (silver side against the eyes) for 10 minutes, according to manufacturer recommendation.[31] The temperature profile of an EyeBag heated for 30 seconds in a 900W microwave is detailed in the Appendix. Digital massage of the eyelids was then conducted according to manufacturer instructions (i.e. a gentle sweeping motion across the eyelids in a nasal to temporal motion for 10 cycles). The Current Symptoms Questionnaire (CSQ) was conducted before and after the EyeBag treatment. The Current Symptoms Questionnaire is a subset of the Dry Eye Questionnaire and had been modified to assess immediate symptoms.[44,45] Participants randomized into the control group did not receive the EyeBag

treatment and was asked to complete the CSQ twice, 10 minutes apart, with no intervention in between. Both high and low contrast visual acuity was recorded again immediately after EyeBag treatment.

At the end of the baseline visit, participants in the EyeBag group were provided the EyeBag to use at home. They were instructed to heat the EyeBag in a microwave oven for 30s before placing it over their eyes for 10 minutes. Participants were also instructed to conduct digital massage immediately after the 10 minutes had elapsed. They were not restricted to when they were required to use the EyeBag at home, however they must use it twice a day. Participants in the control group did not receive an EyeBag and were asked to remain on their own dry eye regimen (if applicable) for the duration of the study.

The CSQ was also administered at home via smartphone using the MetricWire app (Kitchener, Ontario, Canada).[46] This app enabled participants to receive notifications and fill out the CSQ on their smartphone. Similar to above, participants who were randomized into the EyeBag group were instructed to fill in the at-home CSQ via smartphone prior to using the EyeBag and then immediately after using the EyeBag. Participants in the control group were asked to fill out the at-home CSQ via smartphone twice, 10 minutes apart, with no intervention in between. Participants were only required to do this once during weeks 1 and 3, so that there would be 2 pairs of pre/post at-home CSQ values per participant.

6.3.4 Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA). Normal data distribution testing was conducted using the Shapiro-Wilk normality test at the level of $\alpha=0.05$. Parametric data is presented as mean±SD, whereas nonparametric data is presented as medians and inter-quartiles (Q1 and Q3).

To determine statistically significant change over time, repeated measures ANOVA with Bonferroni correction and Friedman test with Dunn's multiple comparisons test were used. Paired t-test and Wilcoxon test were used to determine differences between pre and post EyeBag CSQ, and HCVA/LCVA values.

Level of statistical significance was set at $p < 0.05$. Data from only the left eye (where applicable) were analyzed.

6.4 Results

6.4.1 Participants

Twenty five participants completed the study (mean age 38 ± 15 years, 7 male). Twelve were randomized into the EyeBag group (mean age 38 ± 15 years, 3 male), 13 were randomized into the control group (mean age 37 ± 15 years, 4 male). Four participants were found to be ineligible for the study at the screening visit. Their data were not used in the analysis.

6.4.2 Adherence to Therapy

Participants were instructed to use the EyeBag twice daily for 10 minutes each. On average, participants in the EyeBag group reported having used the EyeBag 1.9 times a day, for 9.6 minutes each, from baseline to week 2. From weeks 2 to 4, participants used the EyeBag 1.7 times per day for 9.6 minutes each. Overall, participants in the EyeBag group reported having used the EyeBag 1.8 times per day for approximately 9.6 minutes for the duration of the treatment period.

6.4.3 Clinical Results

There was a significant change in OSDI over time for the treatment group (Figure 6-1, left). For the EyeBag group, the mean score at baseline was 39.1, which decreased significantly (-12.3 points, $p = 0.03$) to 26.8 at week 2 and remained lowered for the duration of the study (change of -12.5 at week 4, $p = 0.03$; and -11.4 at week 8, $p = 0.06$). For the control group, the OSDI score was 41.3 at baseline and did not change significantly over time. Symptom reduction was also reflected in the SANDE score (Figure 6-1, right). The EyeBag group showed a reduction over time from baseline, with the maximum reduction observed at week 4 (-12.9 points). The control group also showed change over time, however in the opposite direction.

Baseline control SANDE score was 52.4 and increased to 63.0 over the duration of the study. None of these changes were statistically significant.

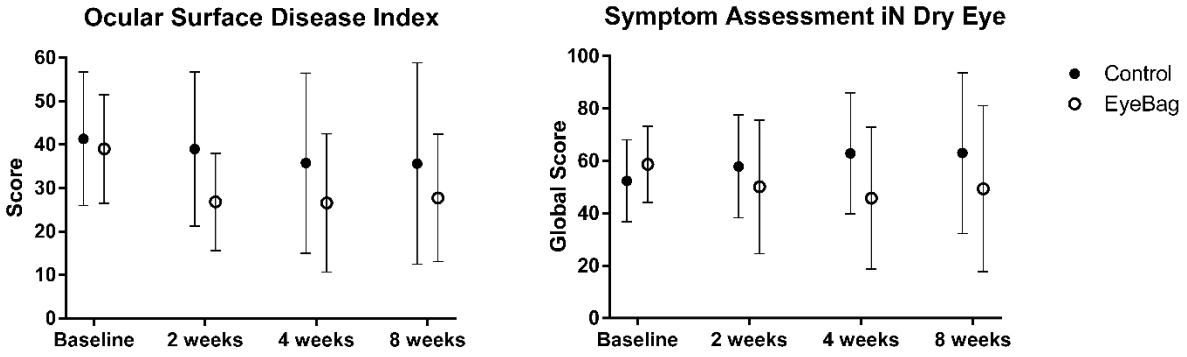


Figure 6-1: Summary (mean±SD) of OSDI (left) and SANDE (right) changes over time. The OSDI scores in the EyeBag group was significantly different than baseline at weeks 2 (-12.3, p = 0.03) and 4 (-12.5, p = 0.03), but not 8 (-11.4, p = 0.06). There was no significant change over time for the control group. There was no significant difference over time in either the EyeBag or control group for the SANDE scores.

The median MG score for the EyeBag group increased from 4.0 to 6.0 points from baseline to week 2, 5.0 at week 4, and returned to 4.0 at week 8. None of these changes were statistically significant. For the control group, the median MG score stayed at 3.0 from baseline to week 2. This decreased to 1.0 at weeks 4 and 8. The changes were also not statistically significant (Figure 6-2, left). The median EyeBag MGYLS count increased from 1.5 at baseline to 2.0 at weeks 2 and 4, and decreased to 0.0 at week 8. In the control group, median MGYLS count stayed at 0.0 for the entire study duration (Figure 6-2, right). None of the changes in either group were statistically significant.

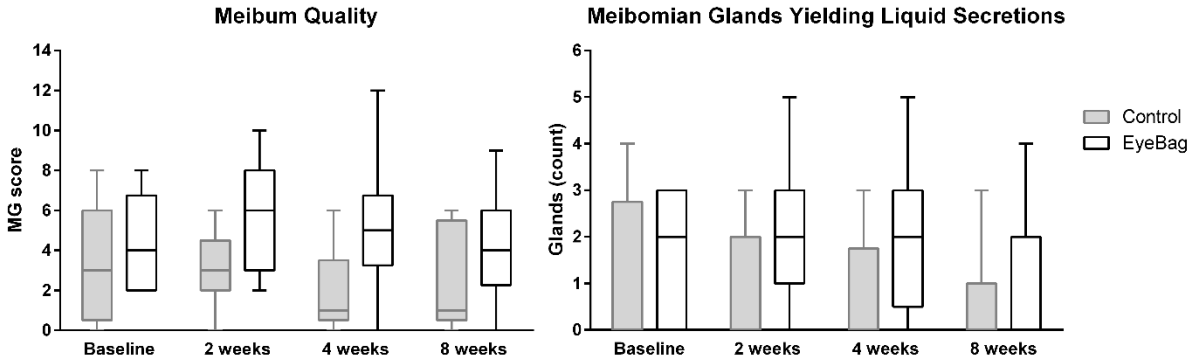


Figure 6-2: Box-and-whisker plots summarizing change in MG score (left) and MGYLS (right). An overall improvement in median meibum quality and glands yielding liquid secretions was observed by 4 weeks relative to baseline (+1.0 units, +0.5 glands, respectively) and appeared to return to baseline levels at week 8. None of these changes were statistically significant. There was no significant change over time in the control group.

For NIBUT, a gradual increase was observed in the EyeBag group. At baseline, NIBUT was observed to be 2.9s. This value increased to 3.1s at 2 weeks, and 3.4s at 4 weeks, before decreasing to 3.0s at week 8. In the control group, NIBUT decreased from 3.1s at baseline to 2.7s at week 2, 2.3s at week 4 and 3.1s at week 8. Neither groups showed significant changes ($p > 0.07$). Figure 6-3 summarizes the change in NIBUT.

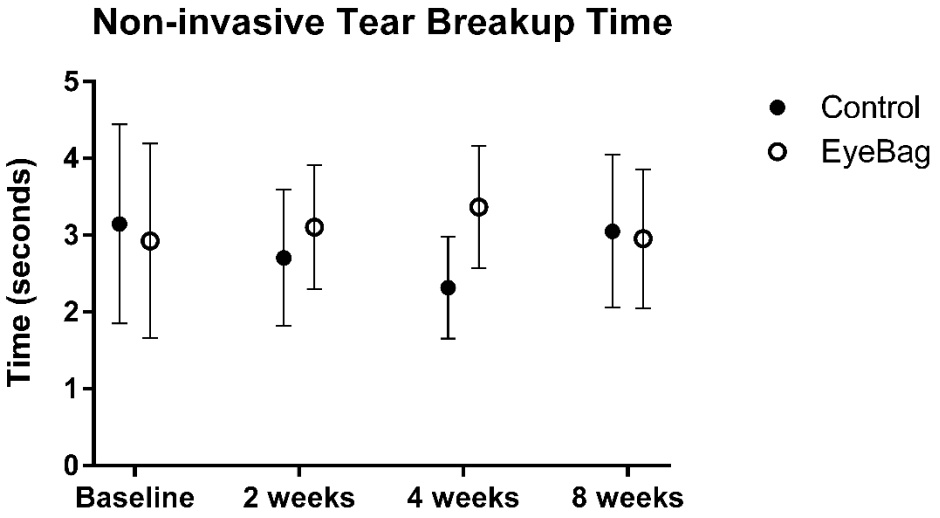


Figure 6-3: Non-invasive tear breakup time (mean±SD) in the EyeBag group increased gradually from baseline to 4 weeks (+0.5s, $p > 0.05$) but returned to baseline levels at week 8. None of the changes in the EyeBag or control group were statistically significant.

There was a statistically significant difference detected in conjunctival staining over time, however it is not likely to be clinically significant. A reduction of 4.0 points on a scale of 100 was relatively minor. Furthermore, there was no significant change in corneal staining, lipid layer thickness, and meibography for both the EyeBag and control group. Tables 6-2 and 6-3 summarize the clinical results for the EyeBag group and control group respectively.

The pre and post EyeBag CSQ scores conducted at CCLR were pooled from all study visits for analysis (n=48 for the EyeBag group, n=52 for the control group). The EyeBag and control group both showed a significant decrease in CSQ scores (-5.5 points for the EyeBag, -2.5 points for the control, both $p < 0.01$ by Paired t-test) over the course of 10 minutes. The decrease in symptoms in the control group was likely describing recovery from discomfort induced by clinical testing. After applying an offset (+2.5 points) to the post-EyeBag CSQ values to correct for clinically induced discomfort, the decrease in symptoms detected in the EyeBag group was still statistically significant ($p < 0.01$). There was no change for both HCVA and LCVA in both groups (Table 6-4).

Participants in the EyeBag group were instructed to complete the at-home CSQ immediately prior to and after the EyeBag treatment (10 minutes). With n=12 in the EyeBag group we had expected a total of 24 pairs (pre/post) of responses (12 pairs for week 1, and another 12 for week 3). Due to some participants who had not completed the at-home CSQ properly, only 19 pairs of data were useable (9 pairs from week 1, 10 pairs from week 3). The median time between the submission of the first at-home CSQ, and the initiation of the second at-home CSQ was 22.6 minutes (Q1: 13.5 mins, Q3: 61.6 mins), ranging from 0.6 mins to 289.1. The median reduction in at-home CSQ scores reported was 5.0 (Q1: 0.0, Q3: 9.0), ranging from -1.0 to 21.0. There was no significant correlation between treatment time and CSQ score reduction (Spearman's rho = 0.14, p = 0.55).

In the control group, of the 26 pairs of responses, only 13 pairs of data were usable. The median time between at-home CSQ submissions was 13.5 minutes (Q1: 9.1 mins, Q3: 22.7 mins) ranging from 1.3 mins to 37.4 mins. The median reduction in at-home CSQ scores reported was 0.0 (Q1: -3.0, Q3: 1.0), ranging from -6.0 to 3.0.

The mean reduction in at-home CSQ scores in the EyeBag group was significantly greater than the control group ($p < 0.05$ by Mann Whitney test).

Table 6-2: Summary of clinical changes for the EyeBag group (n = 12).

	Baseline	**2 weeks**	**4 weeks**	**8 weeks**	**p value**
Parametric (mean ± SD)					
OSDI	39.1 ± 12.5	26.8 ± 11.2*	26.6 ± 15.9*	27.7 ± 14.6	0.02
SANDE	58.7 ± 14.5	50.1 ± 25.5	45.8 ± 27.1	49.4 ± 31.7	0.25
LLT	68.7 ± 16	68.2 ± 14	64.4 ± 11	64.0 ± 13	0.13
NIBUT	2.9 ± 1.2	3.1 ± 0.8	3.4 ± 0.8	3.0 ± 0.9	0.46
Meibography	2.2 ± 1.4	2.3 ± 1.5	2.2 ± 1.3	2.2 ± 1.4	0.53
Non parametric (Q1, median, Q3)					
MG Score[α]	2.0, 4.0, 6.0	3.0, 6.0, 8.0	3.2, 5.0, 6.8	2.2, 4.0, 6.0	0.21
MGYLS[α]	0.0, 1.5, 3.0	1.0, 2.0, 3.0	1.0, 2.0, 3.0	0.0, 0.0, 2.0	0.14
Corneal staining[α]	3.0, 11.2,107.5	2.5, 25.0, 87.5	2.5, 62.5, 137.5	0.0, 62.5, 118.8	0.95
Conjunctival staining[α]	2.6, 4.0, 10.4	0.0, 0.0, 1.1*	3.8, 6.3, 13.4	0.0, 0.0, 2.2*	<0.01

[α] indicates Friedman Test
[*] indicates $p < 0.05$ from baseline

Table 6-3: Summary of clinical changes for the control group (n = 13).

	Baseline	2 weeks	4 weeks	8 weeks	p value
Parametric (mean ± SD)					
OSDI	41.3 ± 15.4	39.0 ± 17.8	35.8 ± 20.7	35.6 ± 23.2	0.23
SANDE	52.4 ± 15.6	57.9 ± 19.6	62.9 ± 23.1	63.0 ± 30.7	0.15
NIBUT	3.1 ± 1.3	2.7 ± 0.9	2.3 ± 0.7	3.1 ± 1.0	0.08
Meibography	2.2 ± 1.6	2.2 ± 1.6	2.2 ± 1.4	1.8 ± 1.4	0.16
Non Parametric (Q1, median, Q3)					
MG Score$^{\alpha}$	0.5, 3.0, 6.0	2.0, 3.0, 4.5	0.5, 1.0, 3.5	0.5, 1.0, 5.5	0.16
MGYLS$^{\alpha}$	0.0, 0.0, 2.5	0.0, 0.0, 2.0	0.0, 0.0, 1.5	0.0, 0.0, 1.0	0.40
LLT$^{\alpha}$	43.5, 65.0, 76.0	49.0, 59.0, 71.0	51.0, 52.0, 56.0	48.0, 51.0, 56.5	0.07
Corneal staining$^{\alpha}$	0.0, 10.0, 25.0	0.0, 25.0, 50.0	0.0, 0.0, 25.0	0.0, 0.0, 37.5	0.37
Conjunctival staining$^{\alpha}$	0.0, 1.2, 5.2	0.0, 0.0, 1.9	0.6, 2.2, 13.8	0.0, 0.0, 1.2	<0.01

$^{\alpha}$ indicates Friedman Test
* indicates $p < 0.05$ from baseline

Table 6-4: Paired t-test comparison of pooled visual acuities and at-CCLR CSQ scores immediately pre and post EyeBag (after offset) application.

	EyeBag group (n = 48)			Control group (n = 52)		
	HCVA	**LCVA**	**CSQ**	**HCVA**	**LCVA**	**CSQ**
Pre Eyebag	-0.04±0.10	0.21±0.10	15.0±8.0	0.00±0.12	0.31±0.17	17.3±8.4
Post Eyebag	-0.05±0.10	0.19±0.12	12.0±6.2	0.01±0.12	0.29±0.17	17.3±8.4
p value	0.11	0.11	<0.01	0.82	0.09	1.00

6.5 Discussion

The results from this study suggest that the changes in terms of measurable signs from using the EyeBag are modest. A one point increase in MG score from baseline to the second week suggests that, on average, one of five glands showed an improvement in meibum grade. This change is maintained up to the fourth week but returned to baseline after discontinuing the EyeBag. This trend is also observed with NIBUT, where an improvement was observed during the period in which the EyeBag was used, returning to baseline where it was discontinued. This suggests that the EyeBag needs to be used continuously to maintain the improvement in MG function and NIBUT. The potential changes in such clinical measures after extended use remains unknown.

The statistically non-significant findings with MG score and NIBUT was likely due to an over-estimation of the impact of the EyeBag when calculating sample size. A post-hoc analysis showed that the effect size of the MG score in this study was actually around 0.4, which was much less than what the study was powered to detect (1.0). One reason that may explain a depressed effect size could be related to the dry winter environment during which this study was conducted. Therefore, to detect a statistically significant difference, future studies using this same study design and analysis would require at least n=52 per group. The other EyeBag study[28] that found a statistically significant improvement with the EyeBag with sample size of n=25 had used a contralateral-eye design which allowed for more statistical power to detect smaller differences.

Despite modest changes in MG score and NIBUT, both short (CSQ) and long-term (OSDI) symptoms improved significantly after using the EyeBag. Long-term improvement was noticed as soon as week 2, and the OSDI remained decreased for the duration of the study, even after the EyeBag was discontinued. It is not clear why this is the case, since both MG score and NIBUT appeared to return to baseline after discontinuing use. Perhaps there exists a lag period after which symptoms are reflected in the change in function. Alternatively, there may exist a placebo effect,[47] in which participants believe they feel better after having undergone the EyeBag therapy. In either case, the OSDI score reduction was not as high as reported by the previous study[28] and we believe the difference is due to bias inherent with a contralateral design and the application of the questionnaire in a unilateral manner.

Short term symptom reduction measured with the CSQ was also considered to be minor. A 3.0 point (at-CCLR) and 5.0 point (at-home) CSQ score reduction corresponds to a 6% and 10% improvement. It was also not possible to predict symptom improvement as there appears to be no observable correlation between treatment time and CSQ symptom reduction.

Meibomian gland atrophy did not change significantly throughout the study. This was not surprising, since it takes many years for MG atrophy to occur.[43] Due to this fact, we also cannot conclude whether or not the

EyeBag prevents or reverses atrophy. Furthermore, we did not find any clinically meaningful changes with corneal staining, conjunctival staining and lipid layer thickness.

Due to the importance of adherence in medical therapy[48] this study had attempted to quantify EyeBag use with two methods (self-reporting to research assistant, and via MetricWire app). Although participants self-reported using the EyeBag 1.8 times a day for 9.6 minutes, the timestamps from the CSQs administered via the MetricWire app suggested differently. We could only conclude that participants were either not completing the CSQs in the instructed manner or they were using the EyeBag differently from what they reported. At the end of the study, some participants mentioned they had fallen asleep while using the EyeBag, while some others mentioned that 10 minutes was too time consuming. Without any more information, it is not possible to make any further conclusions with EyeBag compliance.

All participants have different microwave ovens so it was difficult to determine if all EyeBags were heated to the same extent. There also appears to be variation in EyeBag temperature retention between EyeBags (Appendix) after being heated with the same amount of power. The difference in temperature profiles may be due to differences in which the EyeBag was positioned in the microwave. There may also have been residual heat on the table after a previous measurement that may have impacted overall temperatures.

The EyeBag is aimed towards managing obstructive MGD by melting and softening meibum obstructing the MGs. However, various forms of MGD exist, and not necessarily all driven by obstruction[49] and it is currently unclear whether or not atrophied glands may benefit from the EyeBag treatment. This may be an area for future research. Additionally, in severe cases of MGD, eyelid warming devices may not be enough for treatment. Blepharitis is often associated with MGD[50] and it may be beneficial to study the combined effect of an eyelid warming device with antibiotics or anti-inflammatory therapies. Additionally, since there are many emerging eyelid warming devices, it may also be beneficial to compare their efficacies in the management of MGD.

6.6 Conclusion

There was a considerable improvement in long and short term symptoms after using the MGDRx® EyeBag, with only modest improvement in MG function and tear breakup time. The continued use of the Eyebag is required to sustain improvement, but given that symptoms did improve, then compliance may be helped by the fact that subjects do seem to appreciate a reduction in symptoms.

7 The Effect of Lid Debridement-Scaling in Sjögren's Syndrome Dry Eye

This chapter is published as follows:

Ngo W, Caffery B, Srinivasan S, Jones LW. Effect of Lid Debridement-Scaling in Sjogren Syndrome Dry Eye. *Optom Vis Sci.* Sep 2015;92(9):e316-320.

	Concept & Design	Recruitment	Acquisition of Data	Analysis	Write-up/publication
Ngo	Y	Y	Y	Y	Y
Caffery		Y			Y
Srinivasan	Y				Y
Jones	Y				Y

Table detailing role of each author in this publication (Y denotes significant contribution).

7.1 Overview

PURPOSE**:** To evaluate the effect of lid debridement-scaling (LDS) on dry eye signs and symptoms in individuals with Sjögren's Syndrome (SS).

METHODS**:** This prospective randomized controlled study enrolled 14 female participants with SS. Seven participants were randomized into the treatment group where they were selected to receive LDS, the remainder did not receive LDS and served as controls. LDS was conducted using a stainless steel golf club spud (Hilco Wilson Ophthalmics, Plainville, MA) on both the upper and lower eyelids of both eyes. Outcome variables were assessed prior to LDS and again 1 month later. The outcome variables were the Ocular Surface Disease Index (OSDI), Symptoms iN Assessment of Dry Eye (SANDE) visual analogue scores, ocular staining (SICCA OSS) fluorescein tear breakup time (FLBUT), meibomian gland score (MG score), meibomian glands yielding liquid secretions (MGYLS), and Line of Marx's (LOM) position.

RESULTS**:** Thirteen participants completed the study. Data from the right eye only were analyzed. For the control group (n=6, mean age=62.3±11.6), the pre LDS, post LDS, and significance level (pre mean±SD vs post mean±SD; p-value) were: OSDI (58.3±22.1 vs 48.3±29.0; p=0.051), SANDE (77.4±22.1, 89.6±32.6, p=0.20), SICCA OSS (7.0±4.5 vs 8.2±3.5; p=0.25), MG score (1.3±1.5 vs 1.0±0.9; p=0.75), MGYLS (0.3±0.5 vs 0.0±0.0; p=0.50), FLBUT (2.99 ±1.54 vs 2.85±1.79; p=0.63), LOM (2.0±0.0, 2.0±0.0, p=n/a).

For the treatment group (n=7, mean age=58.0±8.1), the pre LDS, post LDS, and significance level were: OSDI (63.2±13.3 vs 46.9±19.4; p=0.04), SANDE (72.6±17.1, 77.0±28.0; p=0.54), SICCA OSS (6.6±2.9 vs 5.0±3.9; p=0.02), MG score (1.0±1.2 vs 3.1±1.7; p=0.01), MGYLS (0.0±0.0 vs 0.6±1.0; p=0.50), FLBUT (3.13±0.81 vs 3.45±1.03; p=0.53), LOM (0.9±0.9, 1.0±1.0, p=1.00).

CONCLUSIONS**:** This pilot study showed that LDS improved symptoms, ocular staining and meibomian gland function for the group that received LDS. This indicates that LDS can aid in the management of SS dry eye.

7.2 Introduction

Dry eye is a multifactorial disease characterized by symptoms, not limited to stinging, grittiness and or burning sensation of the eyes.[1] The Dry Eye Workshop 2007 defined dry eye as follows[1]: "*Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface".*

Aqueous deficiency is one of two major classifications for dry eye and a major cause for aqueous deficiency is Sjögren's Syndrome (SS).[1] SS is an autoimmune condition in which lymphocytes infiltrate and attack the lacrimal gland, causing damage to the gland tissue.[2] The American College of Rheumatology had proposed an expert consensus approach to the diagnosis of SS, which communicates a positive diagnosis if two of the three following criteria are met[3]:

1. Positive serum anti-SSA/Ro and/or/anti-SSB/La (or positive rheumatoid factor and ANA titer ≥ 1:320),
2. Labial salivary gland biopsy exhibiting focal lymphocytic sialadenitis with a focus score ≥ 1 focus/4mm^2,
3. Ocular staining score ≥ 3.

Recent studies have shown that the meibomian glands (MG) are also affected in SS,[4,5] showing functional and morphological changes to their structure. One of the primary mechanisms driving obstruction of the MGs is hyperkeratinization of the eyelid margin and duct orifices.[6] There are various endogenous and exogenous factors that influence keratinization of the duct epithelium, including stem cell migration/differentiation abnormality, medication use and aging.[6] As keratinized material is built up around and within the orifice, the gland is obstructed and meibum cannot be delivered from the gland to the tear film.[6]

In addition, the flow of meibum from the orifice to the tear film can be hindered with the accumulation of debris that is associated with the thickening of the Line of Marx (LOM).[7] The positional change to the LOM may be due to hyperosmolar changes in the tear film.[8,9] A recent study by Korb et al.[7] described a novel procedure termed "lid debridement-scaling" (LDS) that was shown to increase MG function and reduce symptoms by debriding the LOM. This technique works by mechanically removing accumulated debris and keratinized cells from the eyelid margin to increase the flow of meibum into the tear film.[7]

To date, the aforementioned study[7] appears to be the only publication describing the debridement of the LOM and the subsequent improvement in symptoms and MG function. It is currently unknown how effective this technique is at providing relief for individuals who experience extreme dry eye, which typically occurs in SS. Therefore this study aims to determine how effective LDS is at improving clinical signs and symptoms, in addition to MG function, in individuals with SS.

7.3 Methods

7.3.1 Participants

This was a prospective, unmasked, randomized, controlled study that enrolled 14 participants with SS. Eligibility was determined at a screening visit. Diagnosis of SS was confirmed as per the criteria outlined by the American College of Rheumatology.[3] Seven participants were randomly selected to receive LDS, and the remainder served as controls. All participants except one returned one month later for follow up measurements. This study was conducted in accordance with the ethical principles in the Declaration of Helsinki. Ethics clearance was obtained through a University of Waterloo Research Ethics Committee prior to commencement of the study. Informed consent was obtained from all participants prior to enrolment in the study.

7.3.2 Clinical Methods

All measurements were obtained by the same investigator (WN).

To assess symptoms, the Ocular Surface Disease Index (OSDI)[10] and the Symptom Assessment iN Dry Eye (SANDE)[11] 1 & 2 visual analogue scales were used. OSDI and SANDE were conducted at the beginning of each appointment, prior to testing.

Fluorescein break-up time (FLBUT) was conducted by wetting a fluorescein strip (Fluorets, Bausch & Lomb Canada Inc., Markham, Ontario, Canada) with a few drops of saline (Bausch & Lomb Sensitive Eyes® Saline Plus, Rochester, New York, USA), shaking off any excess dye, and then applying the strip to the lower fornix of both eyes. The time for disturbances in the tear film to appear was recorded in seconds using a stop-watch. At this point, corneal staining was also assessed. Lissamine green (GreenGlo™, HUB pharmaceuticals, Rancho Cucamonga, California, USA) was then instilled by wetting a strip with a few drops of saline, shaking off any excess dye, and then applying the strip to the lower fornix of both eyes. The combination of corneal and conjunctival staining was graded using the Sjögren's International Collaborative Clinical Alliance Ocular Staining Score (SICCA OSS).[12] This grading scheme factors in additional clinical features such as staining in pupillary area, presence confluent staining, and presence of filaments, for a total maximum score of 12 per eye.

Meibomian gland score (MG score) was obtained by using the Meibomian Gland Evaluator™ (MGE) (TearScience, Inc., Morrisville, North Carolina, USA)[13] to apply a controlled and constant pressure of approximately 1.25g/mm^2 to the central 5 glands of the inferior lid margin for 10 seconds.[13] The meibum expressed was graded based on the following scale, and was scored out of 15 (5 central glands were evaluated, with a maximum score of 3 for each gland)[13]:

Grade 0: No expression

Grade 1: Inspissated (toothpaste)

Grade 2: Cloudy with debris

Grade 3: Clear

The Meibomian Glands Yielding Liquid Secretions (MGYLS) measurement is a count of those 5 glands with MG score of 2 or higher.[13]

LOM position was determined by instilling an additional drop of lissamine green and everting the lower eyelid margin for observation. This highlighted the LOM and keratinized debris that accumulated on the lid margin. The position of the LOM relative to the MG orifices was graded based on the following scale:[14]

Grade 0: LOM mostly (>75%) posterior to the orifices

Grade 1: LOM mostly bisecting the orifices

Grade 1: LOM mixed posterior and bisecting the orifices

Grade 2: LOM mostly anterior

Grade 2: LOM mixed posterior, bisecting and anterior to the orifices

Grade 2: LOM mixed bisecting and anterior to the orifices

LDS was conducted by instilling a single drop of proparacaine hydrochloride 0.5% (Alcon, Inc., Fort Worth, Texas, USA) in both eyes to reduce lid sensation and any discomfort. The stained cells/debris were gently debrided using a stainless steel golf club spud (Hilco Wilson Ophthalmics, Plainville, MA, USA). The stained cells/debris were debrided by tracking the head of the golf spud gently across the lid margin in both directions (Figure 7-1). In a manner previously described,[7] this procedure did not remove any debris or cells that could not be removed with merely a mild/gentle force.

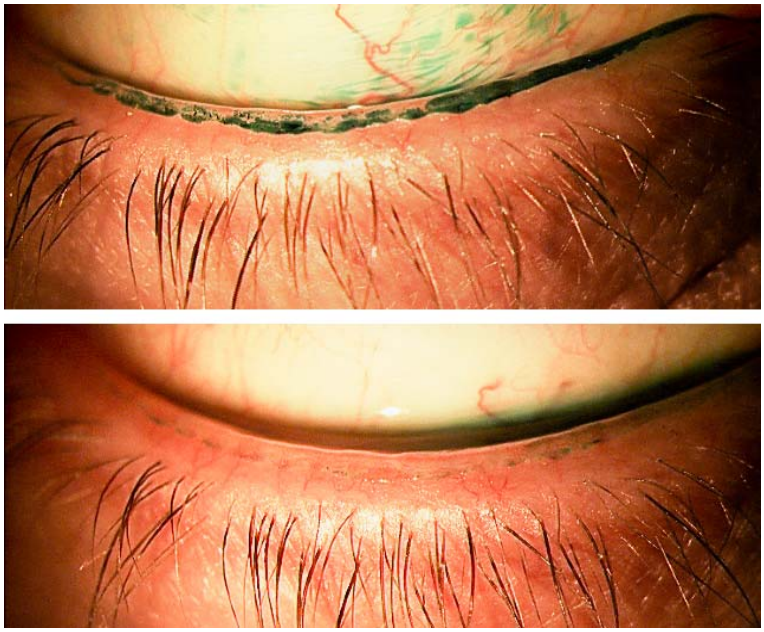


Figure 7-1: Above, the LOM is shown highlighted with lissamine green. Below, LDS had removed stained debris and keratinized cells from the LOM.

7.3.3 Statistical Analysis

Statistical analyses were conducted with GraphPad Prism 6.05 (GraphPad Software, San Diego, CA, USA) and STATISTICA 7.1 (StatSoft, Inc., Tulsa, OK, USA). The normality of distribution was tested using the Shapiro-Wilk test, at the level of $\alpha=0.05$ with STATISTICA 7.1. GraphPad Prism 6.05 was used for the remainder of the statistical tests. Paired t-test was used to determine the statistical significance of the difference of means in parametric ocular variables. Wilcoxon test was conducted on ocular variables that did not pass the normality test. Spearman correlation was examined on all ocular variables and symptoms collected from the baseline visit. Data from only the right eye (where applicable) were analyzed. Level of statistical significance was set at $p < 0.05$.

7.4 Results

7.4.1 Participants

A total of 13 females completed the study (mean age = 60.0±9.7, from 46.0 to 76.0 years). One participant was unable to attend her final visit due to scheduling conflict. Seven participants were randomized to the treatment group (mean age = 58.0±8.1 years), and the remaining 6 were randomized to the control group (mean age = 62.3±11.6 years). The mean ages of the two groups were not significantly different (p=0.46).

7.4.2 Clinical Outcomes

The difference in means between the baseline and 1 month visit, along with statistical significance for each group is summarized in Table 7-1. The treatment group showed a statistically significant reduction in dry eye symptoms and signs with both the OSDI scores (-16.3 points) and in SICCA OSS (-1.6 grade units). Also, the MG score improved by +2.1 grade units. Post treatment FLBUT increased, but not significantly. There was no significant difference in SANDE analogue scale scores, MGYLS, and LOM placement. The control group did not show any significant changes in any of the variables tested.

The Spearman correlation between each variable is summarized in Table 7-2. FLBUT correlated significantly with SICCA OSS and MGYLS. OSDI correlated with SANDE, but this relationship was not statistically significant.

Table 7-1: Summary of difference in means between visits and level of significance for both treatment and control group.

	Control Group			Treatment Group		
Test	**Baseline**	**1 Month**	**p-value**	**Baseline**	**1 Month**	**p-value**
SICCA OSS (0-12)	7.0±4.5	8.2±3.5	0.25[α]	6.6±2.9	5.0±3.9	**0.02***
MG score (0-15)	1.3±1.5	1.0±0.9	0.75[α]	1.0±1.2	3.1±1.7	**0.01***
MGYLS (0-5)	0.3±0.5	0.0±0.0	0.50[α]	0.0±0.0	0.6±1.0	0.50[α]
FLBUT (seconds)	2.99±1.54	2.85±1.79	0.63	3.13±0.81	3.45±1.03	0.53
LOM (0-2)	2.0±0.0	2.0±0.0	----	0.9±0.9	1.0±1.0	1.00[α]
OSDI (0-100)	58.3±22.1	48.3±29.0	0.051	63.2±13.3	46.9±19.4	**0.04***
SANDE (0-100)	77.4±22.1	89.6±32.6	0.20	72.6±17.1	77.0±28.0	0.54

Bold and * indicates significant differences.
[α] indicates Wilcoxon test.

Table 7-2: Spearman correlation matrix displaying the linear relationship between each variable.

	SICCA OSS	**MGS**	**MGYLS**	**FLBUT**	**LOM**	**SANDE**	**OSDI**
SICCA OSS		0.43	0.35	**-0.72**	0.28	-0.28	-0.13
MG score	0.43		0.60	-0.45	0.00	0.37	0.25
MGYLS	0.35	0.60		**-0.34**	0.33	0.23	-0.03
FLBUT	**-0.72**	-0.45	**-0.34**		-0.24	-0.26	0.06
LOM	0.28	0.00	0.33	-0.24		0.06	-0.27
SANDE	-0.28	0.37	0.23	-0.26	0.06		0.55
OSDI	-0.13	0.25	-0.03	0.06	-0.27	0.55	

bold denotes $p \leq 0.05$

7.5 Discussion

This study showed that LDS improved MG function and reduced symptoms in individuals with SS. All participants reported the LDS procedure to be relatively painless and reported that the sensation was similar to a "mild tickling" sensation. Although this study used proparacaine to reduce and avoid discomfort, it did not appear to be necessary and it is possible that many participants would be able to tolerate the procedure without it. We did try the procedure without anesthesia on a few volunteers after the study and minimal discomfort was reported.

Hyperkeratinization of the eyelid margin is one mechanism that may drive obstruction of the MGs, ultimately leading to atrophy of glandular tissue.[6] However, there are other non-obstructive mechanisms

that can also lead to atrophy of MGs, for example inflammation and aging. An *in vivo* confocal imaging study of the MGs in SS showed that individuals with SS had larger amounts of periglandular inflammation compared to non-SS individuals with MGD,[5] and a second *in vivo* study showed presence of glandular atrophy, both in minimal or absent orifice obstruction.[15] We may not yet be able to reverse age-related changes and LDS does not manage the inflammatory aspect of SS related MGD. Thus, it is possible that anti-inflammatory therapy in conjunction with LDS may be helpful in managing MGD in SS.

Improvement in MG function was modest. The function of the MGs was graded with both MGS and MGYLS. The MG score was scored out of 15 (5 central glands were evaluated, with a maximum score of 3 for each gland). Participants typically presented with a score of 1/15, which can be interpreted as "only 1 gland out of the 5 expressed meibum, and the meibum coming out of that gland was opaque with a toothpaste-like appearance". After LDS treatment, the MG score increased on average approximately 2 points, resulting in a total score of 3/15. Thus, it could be interpreted as "of the 5 glands, only 3 expressed meibum, but each of the expressing glands had meibum that was still opaque and toothpaste-like in consistency". This is not an inaccurate description of improvement, since the MGYLS count did not change significantly post LDS. MGYLS is a count of the glands that secreted *liquid* secretions. An increase in MG score but without an increase in MGYLS suggests that the LDS increased the function of MGs by increasing the *quantity* of meibum expressed, without improving the *quality* of meibum. This is in accord with the mechanism behind the LDS technique – which allows for delivery of the meibum by mechanically removing the barrier of keratinized cells and debris obstructing the delivery of meibum onto the ocular surface.[7] A possible next step would be to determine the rate at which eyelid hyperkeratinization occurs and the frequency at which LDS should be conducted.

Meibum helps stabilize the tear film.[16] A previous study showed that it is the *quality* of meibum, not the *quantity* that affects tear film stability.[17] Since this treatment did not aim to improve meibum quality, FLBUT was not expected to be significantly different post LDS, and the findings of this study appeared to fit this expectation. The statistically significant correlation found between FLBUT and MGYLS may be a

false positive result. All of the 13 participants at baseline had an MGYLS count of 0, with the exception of 2 participants, and those 2 participants each had an MGYLS count of 1. Thus, there is not enough information to make a conclusion regarding the correlation between FLBUT and MGYLS.

This study did find a strong correlation between ocular staining and FLBUT (in both pre and post LDS). In previous studies,[18,19] the relationship between FLBUT and corneal and conjunctival staining (separately) was found to be poor. A possible reason to explain this discrepancy may relate to the nature of the SICCA OSS scale. The SICCA OSS grading scale factored in the presence of filaments, which if present would not only increase the SICCA OSS score, but would also quickly reduce the stability of the tear film. In this study, 4 of 13 participants were positive for filamentary keratitis.

In addition to the LOM grading scale used in this study[14] there is only one other LOM grading scale published.[9] The grading of LOM position relative to the MG orifices did not change significantly pre and post LDS. This was expected, since the thickening and advancement of the LOM is a morphological change[8,9] of the eyelid and is not likely to be reversed by LDS.

While the magnitude of change in OSDI (-16.3) was considered to be clinically significant,[20] none of the symptom assessments correlated significantly with any of the clinical signs. This was not surprising, since the relationship between dry eye signs and symptoms is known to be poor.[18,19,21,22] The OSDI showed a positive correlation with the SANDE severity scores, however this relationship was not statistically significant.

Two major limitations of this study include the small sample size and that the treatment was administered without masking of either the subjects or investigators. An issue to note related to this latter point is that the control group's OSDI score also decreased at the 1 month follow-up (Table 7-1). While this reduction was not statistically significantly, the 10 point reduction was near the magnitude of that seen in the treatment group, which reduced by approximately 16 points. This decrease in symptoms is difficult to explain, but could have been due to the "Hawthorne effect"[23] in the control group, in which symptoms change due to

the mere fact that subjects are being observed, regardless of the fact that no treatment is being undertaken. The lack of masking may also have caused the treatment group to have exaggerated their improvement due to a placebo-effect. While this study was still able to show significant improvements with MG score and OSDI and ocular staining, having a larger sample size may have helped us determine whether or not there were improvements in other clinical variables.

Many participants who had LDS reported that their eyelid margins felt a pleasant "cool" sensation for about a week after the procedure, and that their symptoms had improved slightly for a couple of weeks. The cooling sensation could be due to the exposure of the debrided eyelid margin to the environment. The improvement in symptoms may be due to the *belief* that the treatment had helped, and may be unrelated to whether or not it truly did. One way to control for this factor would have been to administer a placebo treatment, in which the LDS treatment was imitated but without actually debriding the eyelid surface and a study using such a sham procedure may be worthy.

7.6 Conclusion

This pilot study was able to show that LDS was effective in improving some clinical signs and symptoms in participants with SS who exhibited severe dry eye and may play a helpful role in the management of SS dry eye.

8 The Relief of Dry Eye Signs and Symptoms Using a Combination of Lubricants, Lid Hygiene, and Ocular Nutraceuticals

This chapter is published as follows:

Ngo W, Srinivasan S, Houtman D, Jones LW. The relief of dry eye signs and symptoms using a combination of lubricants, lid hygiene, and ocular nutraceuticals. *J Optom.* DOI:10.1016/j.optom.2016.05.001.

	Concept & Design	Recruitment	Acquisition of Data	Analysis	Write-up/publication
Ngo	Y	Y	Y	Y	Y
Srinivasan	Y				Y
Houtman	Y				Y
Jones	Y				Y

Table detailing role of each author in this publication (Y denotes significant contribution).

8.1 Overview

PURPOSE**:** To determine the combined effect of TheraTears® Lubricant Eye Drops, TheraTears® SteriLid Eyelid Cleanser, and TheraTears® Nutrition on dry eye signs and symptoms.

METHODS**:** This prospective study enrolled 28 dry eye participants. Participants were instructed to use the Lubricant Eye Drops at least 2-4x a day, SteriLid 1-2x a day, and Nutrition 3 gel caps once a day. Participants were followed up at baseline, 1 month and 3 months. Outcome variables were the Ocular Surface Disease Index (OSDI), Symptom Assessment iN Dry Eye (SANDE) questionnaire, non-invasive tear break-up time (NIBUT), osmolarity, number of meibomian glands blocked (#MG blocked), meibum quality, eyelid margin features, Schirmer's test, tear film lipid layer thickness (LLT), meniscus height, corneal and conjunctival staining.

RESULTS**:** Twenty participants (mean age = 43, from 23 to 66, 17F, 3M) completed the study. Participants reported having used, on average, the Lubricant Eye Drop 2.4x/day, the SteriLid 1.1x/day, and the Nutrition

3 gel caps 1x/day. There was a significant change over time ($p<0.05$) for OSDI (-21.2 points), SANDE (-32.4 points), NIBUT (+0.43s), eyelid margin features (-1.1 grade), meibum quality (-1.0 grade), and #MG blocked (-4.0 glands).

CONCLUSION: By using a combination of TheraTears® Lubricant Eye Drop, SteriLid, and Nutrition, patients experience significant relief in both dry eye symptoms and signs.

8.2 Introduction

Dry eye is a complex multifactorial condition that was defined by the 2007 Dry Eye Workshop as:

"*Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface*".[1]

The two major etiological categories of dry eye are aqueous deficient and evaporative dry eye.[1] The former consists of a wide variety of conditions that result in a deficiency of the aqueous portion of tears (eg typical aqueous deficient dry eye and Sjögren's syndrome).[1] The latter category includes a group of tear film and adnexa anomalies that quicken the evaporative loss of tears from the surface of the eye (eg meibomian oil deficiency).[1]

In either case, the symptoms brought about by ocular dryness can severely affect quality of life.[2-4] Individuals suffering from dry eye may feel significant discomfort during certain tasks, including driving, reading, computer usage, or simply being in an environment with low humidity.[2]

The clinical assessment of dry eye typically include tests that assess subjective symptoms, along with various features of the ocular surface, adnexa and accessory tear glands.[5] The assessment of symptoms is conducted through symptom questionnaires,[5] with some assessing purely symptoms,[6,7] and others combining symptoms with quality of life measures.[8,9] The physical signs of dry eye are commonly assessed with corneal staining, conjunctival staining, tear breakup time, meibomian gland (MG) function, and by undertaking a Schirmer's test.[5] These clinical tests permit determination of the extent of the condition, along with monitoring any improvement of symptoms and signs with the administration of dry eye treatment.

1

8.3 Methods

8.3.1 Participants

This was a prospective study that enrolled 28 dry eye participants. The key inclusion and exclusion criteria is outlined in Table 8-1.

Table 8-1: Inclusion and exclusion criteria for entry into study

Inclusion	**Exclusion**
Between 18-70 years old	Has any active ocular disease (other than blepharitis, MGD, dry eye), infection or allergies
Has read and signed an information consent letter	Is participating in any concurrent clinical or research study
Is willing and able to follow instructions and maintain the appointment schedule	Has known sensitivity to the diagnostic pharmaceuticals to be used in the study
Exhibits symptoms of dry eye for at least 3 months	Has a systemic condition or taking medications that may affect a study outcome variable
Ocular Surface Disease Index (OSDI) of ≥ 23	Has worn contact lenses in the past 5 years
On a non-omega 3 dry eye regimen that consists of instilling artificial tears at least 3 times a week for the past 3 months	Is currently on, or have used omega 3 supplements in the past 3 months
Has an average non-invasive tear breakup time (NIBUT) of ≤5.00s in at least one eye	Is pregnant, lactating, or planning a pregnancy at the time of enrollment as determined verbally
	Has undergone refractive error surgery
	Has taken part in another pharmaceutical research study within the last 30 days
	Has worn contact lenses within the past 5 years
	Is currently using or have used omega 3 supplements in the past 3 monthsf

The study was conducted at the Centre for Contact Lens Research (CCLR), at the University of Waterloo (UW). The study was conducted in conformance with the ethical principles of the Declaration of Helsinki, the ICH guidelines for Good Clinical Practice, the UW Guidelines for Research with Human Participants. Informed consent was obtained from all participants prior to enrollment in the study. Ethics clearance was obtained through a UW Research Ethics Committee prior to commencement of the study.

All participants were screened at the baseline visit to determine their eligibility. Once eligibility was determined, participants were enrolled and baseline measurements were obtained. Participants were then

asked to cease their current dry eye treatment and provided with the TheraTears® Lubricant Eye Drop, TheraTears® SteriLid, and the TheraTears® Nutrition to use, as per label. Details about the study products can be found in Table A.1.

After leaving the CCLR at the baseline visit, participants were asked to start using the products immediately. All participants were contacted at 2 weeks into the study to ensure that adherence to product use was maintained, to monitor adverse events, and to measure symptoms. All participants returned at 1 month and 3 months for follow up measurements.

8.3.2 Clinical Measurements

At the beginning of each study visit, adherence to product and changes to health or medications was documented.

Symptoms were assessed with the Ocular Surface Disease Index (OSDI),[15] and the Symptoms Assessment iN Dry Eye (SANDE).[6] The OSDI is a dry eye questionnaire that quantified dry eye symptoms in the context of visual symptoms, visual tasks, and environmental factors. The SANDE quantified dry eye symptoms by combining two visual analogue scales that separately assessed frequency and severity of dry eye symptoms.[6]

Tear osmolarity was conducted using the TearLab™ Osmolarity System (TearLab™, California, USA).[16] Prior to testing, participants verified that no eye drops were instilled 2 hours prior to arriving at the visit. The tip of the pen was gently touched to the tear meniscus on the temporal lid margin to obtain a reading, as per manufacturer recommendation.

The tear film lipid layer thickness (LLT) was assessed using the LipiView (TearScience®, North Carolina, USA) in primary gaze.[17] The average interferometric color unit (ICU) for each eye was documented.

Tear meniscus height was measured to 0.01mm accuracy using the Keratograph® 5M (OCULUS Inc, Wetzlar, Germany).[18] The built-in software ruler was used to conduct the measurement. The ruler was

drawn from edge of the tear meniscus at the 6 o'clock position of the pupil vertically downward to the edge of the eyelid margin. This was conducted 3 times and the values were averaged.

Non-invasive tear breakup time was conducted by using a corneal topographer.[19] An illuminated placido disc was projected onto the cornea and imaged with an infrared CCD camera in the Humphrey Atlas® Topographer 991 (Zeiss, California, USA). This was conducted by asking participants to hold their eyes open for as long as they could. A stopwatch was used to quantify the time in which distortions began to appear in the reflected placido disc. This measurement was measured to 0.01s accuracy, and repeated three times and then averaged.

Eyelid margin features were examined under a slit lamp. The parameters of interest were erythema, edema, vascularity and telangiectasia. They were each graded and summed to generate a composite eyelid margin score. The grading scale used for each parameter is outlined in Table A.2.

A strip of fluorescein was wetted with a few drops of saline, and was instilled in each eye to assess corneal staining. Corneal staining was assessed using the CCLR scale, which assessed type, depth, and extent of staining on a scale of 0 to 100 each.[20] After 1 minute had elapsed, fluorescein was instilled once more. After waiting for another 3 minutes, the superior eyelid was everted and fluorescein lid wiper epitheliopathy (LWE) was assessed.[21] A strip of Lissamine green was wetted with a few drops of saline and instilled into both eyes to assess conjunctival staining (using the Oxford Scale).[22] After 1 minute, Lissamine green was instilled again. The eyelids were everted after 3 minutes to assess Lissamine green LWE. Both fluorescein and Lissamine green LWE grades were averaged to generate the final LWE grade.[21] Table A.3 outlines further details on LWE grading.

Meibomian gland function was assessed by observing the expressibility and quality of meibum in the inferior central 8 glands. Meibomian gland expressibility was assessed by applying variable digital pressure to the lid margin and estimating the force required to express meibum. Meibum quality was then assessed by applying firm digital pressure to the lid margin and assessing the physical characteristics of meibum

using a 4 point grading scale previously described.[23] The number of blocked glands (out of 8) were defined as ones that did not express liquid secretions.

Meibography was assessed by everting the lower and upper eyelids and imaging the tarsal plate using the Keratograph® 5M.[24] The amount of MG dropout from the upper and lower eyelids were was quantified using a grading scale previously described,[25] and summed.

Schirmer's test was conducted by inserting a Schirmer strip for 5 minutes in the lateral 1/3 of the eyelid margin. Participants' eyes were closed for the duration of 5 minutes. The amount of wetting after this duration was quantified.

A summary of clinical testing and the order in which they were conducted is summarized in Table 8-2.

Table 8-2: Summary of procedures and instruments

Testing order	Procedure	Instrument
1	Compliance and adverse event check	N/A
2	Symptoms assessment	OSDI and SANDE
3	Entrance visual acuity	Electronic logMAR chart
4	Osmolarity	TearLab Osmolarity System
5	LLT	LipiView
6	Tear meniscus height	Keratograph® 5M
7	NIBUT	Atlas® topographer
8	Eyelid margin features	Slit lamp, fluorescein and Lissamine green.
9	Corneal staining, conjunctival staining	
10	LWE	
11	MG function (meibum quality, expressibility, # glands blocked)	
12	Meibography	Keratograph® 5M
13	Schirmer's test	Schirmer's strips
14	Exit visual acuity	Electronic logMAR chart

8.3.3 Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 6.05 (GraphPad Software, California, USA).

Normal data distribution testing was conducted using the Shapiro-Wilk normality test. Repeated Measures ANOVA was conducted on variables that had passed the normality test with a threshold of alpha=0.05. Post-hoc Dunnett's test was used to determine which visit was significantly different from baseline values in parametric distributions. Friedman test was conducted on non-parametric variables that did not pass the normality test. Dunn's test was used to determine which visits were significantly different from other visits in non-parametric distributions.

Data from only the left eye were analyzed. Level of statistical significance was set at $p < 0.05$.

8.4 Results

8.4.1 Participants

A total of 20 participants (17 female, 3 male) completed the study. The mean age of the participants was 43 (median 41 years, ranging from 23 to 66 years). All participants had previously used lubricant eye drops for at least once a day before switching over to the study products. Participants were not on any dry eye medications (e.g. cyclosporine, steroids) and were not using any eyelid hygiene products at the time. With a combination of OSDI ≥ 23, NIBUT < 5.0s, significantly altered meibum quality and gland obstruction at baseline (Table 8-3), participants in this sample appeared to have moderate to severe dry eye.

8.4.2 Compliance

Participant adherence to product usage was monitored at every visit. On average, participants had used the Lubricant Eye Drops 2.4x per day, SteriLid 1.1x per day, and Nutrition 3 gel caps once daily.

8.4.3 Clinical Outcomes

There was a significant improvement in symptoms as measured by the OSDI (Figure 8-1). The net change from baseline to week 2 (-19.8), to 1 month (-21.4), and to 3 months (-21.2) were all statistically significant (all $p<0.01$). There was also a significant improvement in SANDE scores. The net change at week 2 (-10.2), at 1 month (-21.4), and at 3 months (-32.4) were all statistically significant from baseline (all $p<0.01$).

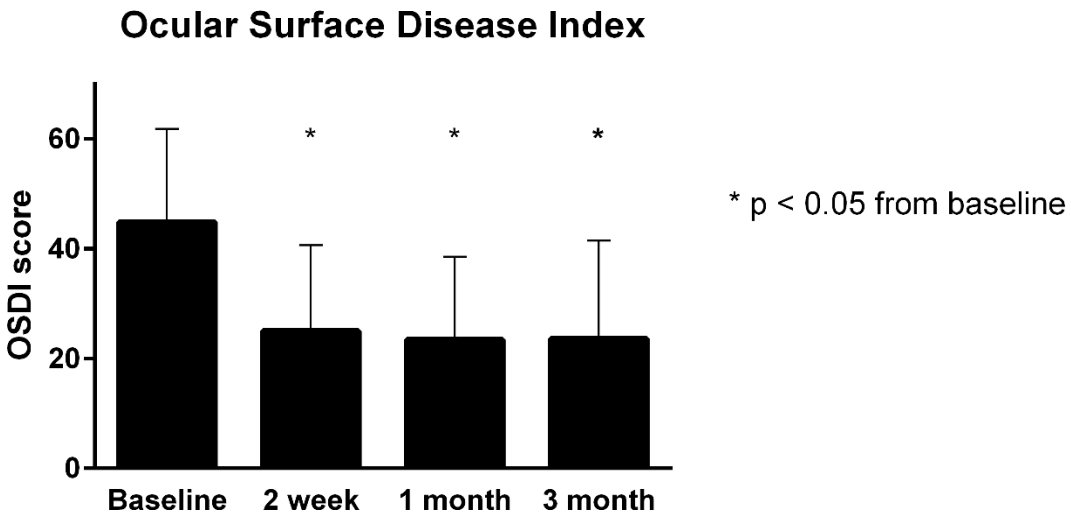


Figure 8-1: The OSDI score showed significant change over time, with a total net change of -21.2 points over the study duration.

NIBUT was significantly improved from baseline. A median improvement of +0.63s at 1 month and +0.48s at 3 months were both statistically significant (both $p<0.05$).

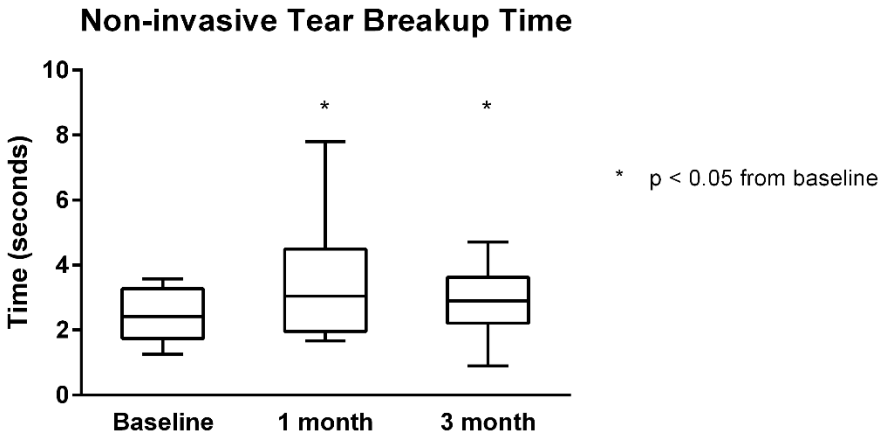


Figure 8-2: NIBUT significantly improved over the course of the study, with a total net change of +0.48s at 3 months.

Eyelid margin scores showed significant change over the course of the 3 months (Figure 8-3). Although the change from baseline to 1 month was not statistically significant (-0.4 grade units, $p>0.05$), the change from baseline to 3 month was significant (-1.1 grade units, $p<0.05$).

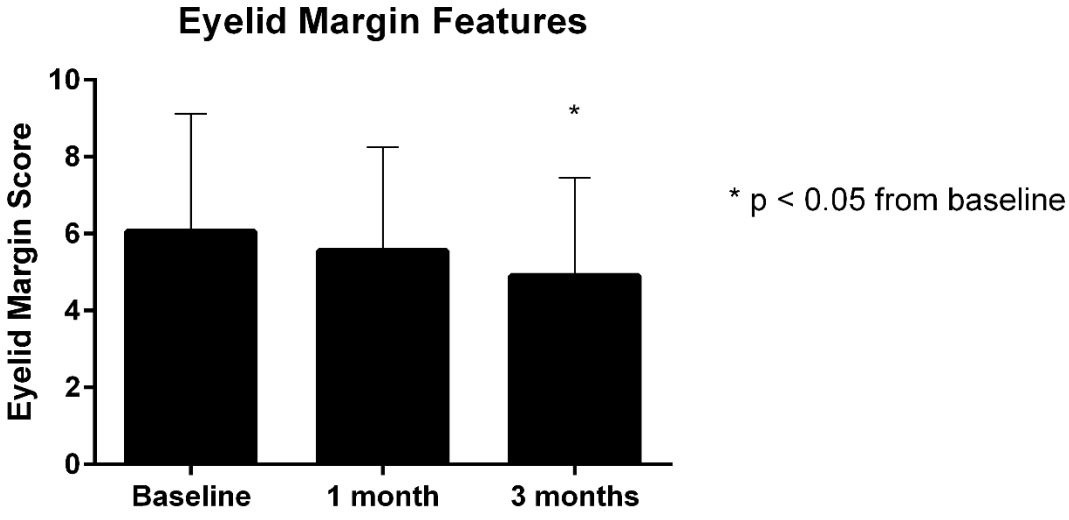


Figure 8-3: Eyelid margin features gradually improved over the course of the 3 months, with a significant net change in of -1.1 grade units from baseline.

Meibomian gland function was also observed to improve significantly (Figure 8-4). Meibum quality was not significantly different than baseline at 1 month, but became significantly different at 3 months (-0.5 grade units, p=0.16; -1.0 grade units, p=0.01, respectively). The number of glands blocked also reduced significantly from baseline to 1 month (-2.0 glands, p=0.04), and to 3 months (-4.0 glands, p<0.01).

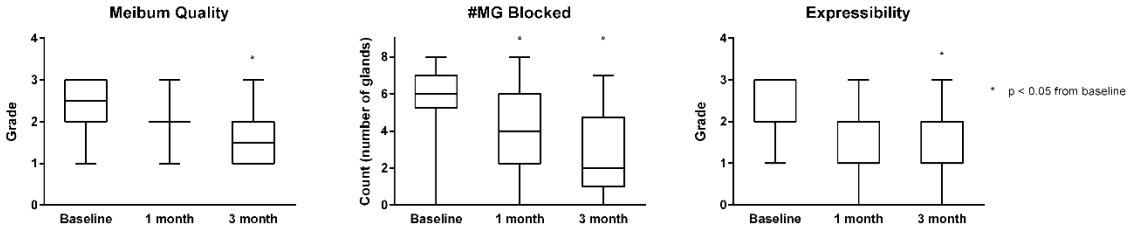


Figure 8-4: Summary of changes to MG function over the course of the study. By the end of 3 months there was a significant improvement from baseline in meibum quality, number of MGs blocked, and expressibility of glands (all p<0.05).

There was no significant difference in Schirmer's test, LLT, tear meniscus height, LWE, corneal staining, conjunctival staining, meibography, and osmolarity. A summary of the clinical results is listed in Table 8-3.

Table 8-3: Summary of clinical changes over time (n=20).

Ocular Measurement	Baseline	2 weeks	1 month	3 month	p-value
Parametric (mean±SD)					
OSDI	44.8 ± 17.0	25.0 ± 15.6*	23.4 ± 15.1*	23.6 ± 17.9*	**<0.01**
SANDE Global Score	63.0 ± 20.6	52.8 ± 23.2*	41.6 ± 27.3*	30.6 ± 25.1*	**<0.01**
Eyelid margin score	6.0 ± 3.1	N/A	5.6 ± 2.7	4.9 ± 2.6*	**0.02**
LLT	82.2 ± 15.4	N/A	76.6 ± 15.9	79.5 ± 16.7	0.21
Osmolarity	301 ± 13	N/A	304 ± 11	302 ± 11	0.35
Non-parametric (Q1, median, Q3)					
NIBUT[a]	1.75, 2.42, 3.27	N/A	1.97, 3.05, 4.49*	2.22, 2.90, 3.63*	**0.02**
Meibum quality[a]	2.0, 2.5, 3.0	N/A	2.0, 2.0, 2.0	1.0, 1.5, 2.0*	**<0.01**
Expressibility[a]	2.0, 2.0, 3.0	N/A	1.0, 2.0, 2.0	1.0, 2.0, 2.0*	**<0.01**
Number of glands blocked[a]	5.3, 6.0, 7.0	N/A	2.3, 4.0, 6.0*	1.0, 2.0, 4.8*	**<0.01**
Schirmer's test[a]	4.5, 8.5, 14.0	N/A	4.0, 9.0, 13.0	5.3, 11.0, 25.0	0.41
Meniscus Height[a]	0.17, 0.20, 0.26	N/A	0.16, 0.19, 0.27	0.18, 0.22, 0.24	0.78
LWE[a]	0.00, 0.00, 0.19	N/A	0.00, 0.00, 0.75	0.00, 0.00, 0.50	0.29
Corneal Staining[a]	14, 53, 96	N/A	5, 40, 124	3, 25, 46	0.36
Conjunctival Staining[a]	0.2, 1.0, 2.0	N/A	0.0, 1.0, 1.0	0.0, 1.0, 1.0	0.08
Meibography[a]	0.2, 2.0, 4.0	N/A	1.0, 2.0, 3.8	1.0, 2.0, 4.0	0.66

[a] denotes Friedman test
* p < 0.05 from baseline

A total of 8 participants were prematurely discontinued from the study. There were 2 participants who had experienced adverse events related to study product use. One participant experienced dyspepsia after ingesting the Nutrition gel caps, and the other participant felt significant eyelid discomfort after using the SteriLid. These symptoms were resolved upon cessation of the study product. The remaining 6 participants were found to be ineligible at screening. The data from these participants were not used in the analysis.

8.5 Discussion

This study showed that a combination of lubricant eye drops, lid hygiene, and oral omega-3 supplements was effective in improving moderate to severe dry eye.

Because of the study design, it is not possible to determine from the data how much each separate component contributed to the improvement in dry eye. Due to differences in clinical testing and grading scales it is also very difficult to compare results to other published studies. For example, the oral omega-3 supplements used in this study (450mg eicosapentaenoic acid (EPA)/300mg docosahexaenoic acid (DHA)/450mg alpha linolenic acid (ALA), total omega-3 content of 1200mg daily) have been shown to be effective in reducing dry eye symptoms,[26,27] however the methods for symptoms assessment or reporting were different from this study. Two previous studies reporting OSDI outcomes using oral omega-3 supplements showed that OSDI scores improved by 11.6 units (participants taking 6000mg flaxseed oil daily),[28] and 8.3 units in a separate study (participants taking 360mg EPA, 240 DHA daily).[29]

The scenario is similar for the lubricant eye drop and the eyelid hygiene product used in this study. This is the first clinical study documenting the effectiveness in relieving symptoms using the TheraTears® lubricant eye drop in conjunction with other dry eye treatments. However, there are no studies with TheraTears® lubricant eye drops as a stand-alone product documenting symptom relief. In other studies that have reported OSDI outcomes with other artificial tears, one had reported a change of approximately 14.0 units with three separate artificial tear drops formulations (used 2-3 times daily) each,[30] and another

study showed that OSDI improved by approximately 13 units with 4 different formulations (used 3 times daily) each.[31] Similarly, the only study that reported an OSDI outcome with an eyelid hygiene product (Blephaclean twice a day) showed an improvement of 10 units.[12]

If we follow the assumption that artificial tears, eyelid hygiene, and omega-3 supplements provide an improvement to OSDI of 13, 10, and 10 points respectively, then a complete additive effect of combined therapy would yield a theoretical improvement of 33 points to the OSDI. However, the differences between reported therapy ingredients, duration of therapy, dosage, population sampling, and study design make it very difficult to estimate the true potential improvement for OSDI scores and it is unlikely that these benefits are summative in this manner. The total improvement in OSDI score in this study (21.2) suggests that combination therapy is approximately twice as effective as reported single therapies in relieving symptoms. Although we have not examined the effectiveness of the single therapies in this combination, it is unlikely that any single product used here could be responsible for an improvement in OSDI of this magnitude. Therefore, an additive effect from at least two of the therapies is likely the case.

The improvement in eyelid margin scores suggests that the combination therapy had an effect in relieving blepharitis. The decrease in clinical inflammation can likely be attributed to the actions of the oral omega-3 supplements, eyelid hygiene, and even the lubricant eye drops. Oral omega-3 supplements have been studied extensively and have been shown to reduce inflammatory biomarkers in the body.[32] The antimicrobial activity of SteriLid against the eyelid bacteria strains have previously been studied in vitro (and compared with povidone iodine).[13] A combination of omega-3 supplements and eyelid hygiene together have been previously studied,[33] and have shown improvements in tear break up time, MG expression, eyelid margin inflammation, and symptomatic relief. These findings are mirrored very well by our study, as we also found significant improvements in MG function, tear breakup time, eyelid margin inflammation and symptoms.

Despite improvements in MG function, there was no significant change in gland atrophy (meibography) over time. This was an expected finding, as MG atrophy occurs at a very slow rate and may take many

years for any change to be detectable. A previous study by Arita et al.[25] documenting the prevalence of age-related MG atrophy showed that changes to MG atrophy can take decades to occur. Therefore, any change (if present) could not have been detected within the course of this study. However, it would be helpful to run a prospective longitudinal study spanning several years to see whether or not adding an intervention can impact gland atrophy rates.

This study was not able to detect any changes in corneal and conjunctival staining. The low amounts of corneal and conjunctival staining presenting at baseline could be due to the fact that participants were already on drops when they presented for this study. Any improvement (if present) from the treatment effect would have been very small, and therefore hard to detect. For future reference, it may be a good idea to consider having participants go on a "washout" period prior to beginning a study such as this, to allow them to manifest their full corneal and conjunctival staining at baseline.

Osmolarity also did not change throughout the study period. Osmolarity is considered to be a complex aspect of dry eye disease involving the breakdown of homeostatic mechanisms.[34] Similar to some of the other measures, the osmolarity readings may have been impacted by the participants presenting at baseline already on drops. A "washout" period prior to the baseline osmolarity reading would have been expected to provide higher initial readings. The 2007 Dry Eye Workshop defines dry eye as high osmolarity readings for participants with dry eye.[1] It would be expected that those participants with high osmolarity readings using the lubricant eye drop in this study containing a hypo-osmolarity component would have decreased osmolarity over time. A previous study showed that higher variability was attributed to blepharitis and Sjögren's syndrome dry eye compared to normals.[35] In our study, we had found that the standard deviations in our osmolarity measurements remained similar over time (12.6 at baseline, 11.1 at 1 month, 11.1 at 3 months) even though we observed improvements in many other areas (eg OSDI, NIBUT, eyelid margin scores). One possible reason for this is that participants in this sample did not exhibit high osmolarity to begin with, therefore making it appear that undergoing treatment had no effect over time.

A limitation of this study was that since there were no placebo controls, a placebo effect may be present and cannot be ruled out. For future work, implementation of an independent control group would help us better understand the findings in this study.

8.6 Conclusion

The combined therapy of TheraTears® Lubricant Eye Drops, TheraTears® SteriLid, and TheraTears® Nutrition improved both symptoms and a variety of signs in participants with moderate to severe dry eye.

9 General Discussion and Future Work

9.1 Discussion

The past few decades have seen vast improvements in meibomian gland (MG) imaging technology. Meibography initially started with crude eyelid transillumination,[1] but has now progressed to the point where MGs can be observed with optical coherence tomography (OCT).[2] In Chapter 3, only the Keratograph 5M (OCULUS, Wetzlar, Germany) was found to successfully image the MGs. The Heidelberg Retinal Tomograph 3 with the Rostock Cornea Module (HRT3/RCM) (Heidelberg Engineering GmbH, Heidelberg, Germany) confocal microscope imaged structures that resembled dermal structures, and the ultra-long OCT was unable to obtain images of MGs. Successful imaging of the MGs with OCT likely requires a central wavelength of at least 1300nm, as demonstrated by existing studies.[2-5] One of the limitations inherent with confocal microscopy is the rapid decrease of signal-to-noise ratio with increasing penetration depth. Modifying the microscope with a longer wavelength laser may reduce scattering and allow viewing of the MG structures.[6]

A comparison between the K5M and the older Keratograph 4 (OCULUS, Wetzlar, Germany) in Chapter 4 showed that the two devices exhibited a low level of agreement and should not be interchanged. The two devices differed in field of view and image contrast outputs, which generated two very different images for interpretation. Examining the repeatability of the 4-point scale yielded an agreement rate of 76%, which was far above the 37% suggested by Bailey et al.[7] for effective clinical grading. In addition, the observers graded within -1 to +1 grade units between and against themselves 95% of the time, which suggested that repeatability of the scale could be improved. After splitting the 4-point scale into half units, yielding a 7-point scale, the agreement rate decreased from 76% to 64%, and the concordance correlation coefficient increased from 0.78 to 0.92. Although this scale was slightly more repeatable, it was still not considered sensitive enough to detect small MG dropout changes. In this chapter, none of the eyelid margin features correlated with MG dropout.

In Chapter 5, a study controlling for age in a female cohort found that only corneal staining, MG quality, number of obstructed MGs, and tear stability were significantly associated with symptoms of dry eye disease. The proposed mechanism involved is that the reduction in MG function resulted in a decrease in tear film stability,[8] which in turn exposed the ocular surface to repeated desiccation. The constant desiccation caused ocular staining[9] and symptoms of dry eye (DE).[10] This mechanism suggested that perhaps MG dysfunction (MGD) was associated with discomfort in the symptomatic group. An interesting observation is that even after controlling for the two major risk factors of age and sex, DE can still be clearly observed in the symptomatic group. Without a complete and entire medical and case history, it was difficult to determine what the exact cause of their MGD was.

Using heat to treat MGD is not a novel idea.[11] The MGDRx EyeBag (The EyeBag Company Ltd, Halifax, UK) is similar in concept to the warm towel compress that has been advocated for many years, however the difference is that the EyeBag can retain heat for a longer period of time.[12] In Chapter 6, the MGDRx EyeBag was not found to have a significant effect on tear film stability and MG function, but did significantly improve long and short term symptoms. It is possible that there was a placebo effect, and that cannot be ruled out without enrolling an extra study arm. In an additional in vitro study, the EyeBags were heated under the recommended duration and microwave wattage/setting (30 seconds @ 900W, maximum power), and found that the maximum temperatures reached was approximately 38^{o}C to 40^{o}C. While this was still within the melting range of meibum, it still fell short of the upper melting range of 45^{o}C.[13] It is possible that the effectiveness of the EyeBag could be improved by heating it for slightly longer than the manufacturer suggested time, but further work is required to investigate this.

Hyperkeratinization of the eyelid margins, leading to stenosis of the MG orifices, is the underlying pathophysiology behind obstructive MGD.[14] The mechanical removal of the debris on the eyelid margin was found to be effective in participants with evaporative DE.[15] In Chapter 7, this technique was applied to patients with Sjögren's syndrome (SS) and found that it was also effective in relieving DE signs and symptoms. This gives clinicians another treatment strategy that can be used to help manage SS DE.

Finally, Chapter 8 of the thesis evaluated a combination approach to managing DE. A combination of a lubricant eye drop, eyelid hygiene, and omega 3 fatty acids was found to be effective at relieving DE signs and symptoms. This combination managed DE in a multi-pronged approach, via lubrication of the ocular surface, removal of bacteria (that metabolized tear film components) and reduction in ocular surface toxins, and the promotion of anti-inflammation therapy. These products are all commercially available and can be readily obtained over-the-counter. However, as there are more products and regimens involved in this combination therapy, the cost for therapy will be higher and patients will be required to take more time to maintain the therapy.

Imaging technology (OCT, confocal microscopy) has the potential to play a larger role in the diagnosis and management of DE. Being able to visualize the structures primarily involved in the disease process can help researchers gain insight into its pathophysiology. Further improving on imaging technology and adapting it for clinical use can then allow clinicians to better monitor DE disease or treatment progress. For this to happen, the fundamentals and limitations of imaging must be first be understood.

However, despite the best imaging technology, human interpretation or subjective clinical grading of videos and images can be a source of error and variability, resulting in a lack of reliability and repeatability. Examining the source of these errors may then help improve or develop grading systems that can accurately and precisely describe a feature of DE presentation (e.g. MG atrophy). This in turn, would improve the testing and diagnosis for DE disease. Currently, the relationship between symptoms and signs is still not understood well. However, by developing better testing methods and technologies, there may be a way to help clarify the relationship between symptoms and signs.

The result of better DE testing would translate to better DE management as well. Smaller variability and better reliability would increase confidence in monitoring treatment values. However, due to the multifactorial nature of DE disease, it would be unlikely that a single DE test could diagnose and stage DE disease alone.

Due to its multifactorial etiology, it is also likely that multiple concurrent treatments may be required to achieve the best outcomes. Different DE etiologies may co-exist with another, e.g. refractive surgery-induced DE and MGD both have different etiologies and would require different management strategies. The treatments explored in this thesis could be combined together to simultaneously manage multiple aspects of DE. The EyeBag could be used in conjunction with lid debridement-scaling to help manage MGD, and the combined lubricant drop, lid hygiene, and omega 3 system could be used to help supplement the tear film and manage inflammation. The most important aspect of these treatments is that they are easily accessible and can be readily used in clinical practice.

9.2 Future Work

The understanding of DE is constantly evolving, and with the completion of this thesis many more areas remain to be explored.

Meibography and MG imaging technology will likely continue to evolve, but the grading scales will need to change to keep up. The grading of MGs with a 4 point scale, or a 7 point scale is still not sensitive enough to detect relatively subtle changes in MG structure. The question as to how many units on a scale would be suitable should be investigated. A way to increase sensitivity of the scale is to increase the amount of grade units on the scale. A researcher may be open to using highly sensitive scales, but they may not be very practical in a clinical setting. A clinician may not likely use, for example, a 23-point scale, no matter how sensitive it is to grade MG dropout. The trade-off with using scales of increased sensitivity is that it takes longer to properly allocate a grade to the clinical feature. Analyzing MG atrophy with imaging processing protocols is promising as it removes the subjective nature of grading. However image processing protocols usually require a defined set of conditions to function optimally, for example, no reflective spots, and that all areas of the eyelid is in focus. Therefore, a development of reliable objective methods will greatly enhance the detection of changes in MG structures.

A problem that is closely related to this is the numerous grading scales that are currently available for grading. For example, there are at least 5 different grading scales for meibum quality,[16] and at least 4 different grading scales for corneal grading.[17] In this thesis, a number of different grading systems were used across different chapters. The reason for this was purely academic in nature, and was intended to be an exercise in learning where experience in using different grading scales could be obtained. While there is currently no consensus or any evidence suggesting a single grading scale that is superior to all others, the multitude of grading scales may cause several problems. Firstly, clinicians may be overwhelmed when they try to find a grading scale to use. Some grading scales call for digital pressure to express the MGs, while others require standardized pressure. There are scales that may be clinically unintuitive (e.g. Korb & Blackie grading scale increases as clinical presentation improves, whereas Mathers et al. decreases), which can make it difficult to adopt. Secondly, the multitude of scales make it difficult to compare results against other clinical studies. Therefore, a possible direction for future work could be to establish a standard for DE testing and grading.

As discussed in Chapter 4, one of the biggest challenges facing MG imaging is that the eversion of the eyelids is not standardized. If the angle of the everted eyelids is not parallel to the imaging plane, MGs can be made to appear or disappear. Developing an eyelid eversion standard will greatly aid clinical grading, but may be difficult to accomplish since eyelids come in many different shapes and forms. There are also no studies that have observed the natural history of MG atrophy in humans. This is an important point to consider where a participant may present with truncated MGs. It is impossible, without observing over time, to know whether this person was born naturally with short MGs, or had developed MG atrophy. Therefore, it is technically incorrect to use the term “MG atrophy” to describe areas without glands, without having observed them first.

The current focus of DE management is not to cure the condition, but rather serve to relieve symptoms of discomfort. With increasing age, the downregulation and senescence of lacrimal structures[18] and associated glands make DE almost an inevitability. There will continue to be iterations of lubricant drops and various

forms of eyelid warming devices, as they remain a mainstay for treatment, and future work to assess their treatment efficacies will be required. Perhaps a different management approach that should be considered is the prevention of DE. It would be valuable to know if there are measures that can be taken to delay, or even prevent, the onset of DE. An example is to determine if conducting regular eyelid hygiene will exhibit any protective effect against developing DE, or if long-term anti-inflammatory supplements are protective against tissue aging. However, these studies will be costly, as it can take several years and hundreds of participants to find any effects, but the results may be extremely valuable and can potentially shift the focus of DE management.

One management aspect that this thesis did not consider, but is vital for future work, relates to environmental stressors (e.g. reduced humidity, pollutants). Environmental factors play a major contributory role to DE disease and are considered to be an important aspect in DE management. Some patients report their symptoms disappearing during the summer months,[19] or as they travel to tropical or temperate climates. As a corollary, it could be possible that some DE treatments have reduced efficacy because they could not overcome the stresses of the environment. Future work should assess the environmental impact on DE treatment efficacy. There could also be studies to determine if there are certain substances in the air that are *beneficial* to reducing DE symptoms. Since DE was shown to impact workplace productivity,[20] it would be in the employers' interest to understand the atmospheric conditions in an office environment to minimize symptoms of DE and minimize productivity loss. This work can potentially have an impact on how workplace policies are developed.

The use of alternative medicine is becoming increasingly popular, as patients wish to be more actively involved in maintaining their health.[21] Some studies have demonstrated the effectiveness of acupuncture[22,23] and abdominal breathing exercises as methods to stimulate tear production.[24] Although alternative medicine is still controversial,[25] it would be amiss to discount this concept completely without critically studying their influence on symptoms and signs of DE. Future work in this area may see clinical trials that study the effectiveness of combined conventional and alternative therapies for the management of DE.

Letters of Copyright Permission

Figure 1-1

ELSEVIER LICENSE
TERMS AND CONDITIONS

May 25, 2016

This is a License Agreement between William Ngo ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited The Boulevard,Langford Lane Kidlington,Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	William Ngo
Customer address	School of Optometry & Vision Science Waterloo, ON N2L 3G1
License number	3876051203228
License date	May 25, 2016
Licensed content publisher	Elsevier
Licensed content publication	The Ocular Surface
Licensed content title	The Definition and Classification of Dry Eye Disease: Report of the Definition and Classification Subcommittee of the International Dry Eye Workshop (2007)
Licensed content author	None
Licensed content date	April 2007
Licensed content volume number	5
Licensed content issue number	2
Number of pages	18
Start Page	75
End Page	92
Type of Use	reuse in a thesis/dissertation
Intended publisher of new work	other
Portion	figures/tables/illustrations
Number of figures/tables /illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No

Will you be translating?	No
Original figure numbers	Figure 1
Title of your thesis/dissertation	Contemporary Diagnosis and Management of Dry Eye
Expected completion date	Aug 2016
Estimated size (number of pages)	200
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 CAD
VAT/Local Sales Tax	0.00 CAD / 0.00 GBP
Total	0.00 CAD
Terms and Conditions	

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.
3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:
"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."
4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.
5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at permissions@elsevier.com)
6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.
7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.
9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.
10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.
11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.
12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).
13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.
14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:
15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article.

16. **Posting licensed content on any Website**: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.
Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com . All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.
17. **For journal authors:** the following clauses are applicable in addition to the above:
Preprints:
A preprint is an author's own write-up of research results and analysis, it has not been peer-reviewed, nor has it had any other value added to it by a publisher (such as formatting, copyright, technical enhancement etc.).
Authors can share their preprints anywhere at any time. Preprints should not be added to or enhanced in any way in order to appear more like, or to substitute for, the final versions of articles however authors can update their preprints on arXiv or RePEc with their Accepted Author Manuscript (see below).
If accepted for publication, we encourage authors to link from the preprint to their formal publication via its DOI. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help users to find, access, cite and use the best available version. Please note that Cell Press, The Lancet and some society-owned have different preprint policies. Information on these policies is available on the journal homepage.
Accepted Author Manuscripts: An accepted author manuscript is the manuscript of an article that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and editor-author communications.
Authors can share their accepted author manuscript:

- immediately
    - via their non-commercial person homepage or blog
    - by updating a preprint in arXiv or RePEc with the accepted manuscript
    - via their research institute or institutional repository for internal institutional uses or as part of an invitation-only research collaboration work-group
    - directly by providing copies to their students or to research collaborators for their personal use
    - for private scholarly sharing as part of an invitation-only work group on commercial sites with which Elsevier has an agreement
- after the embargo period
    - via non-commercial hosting platforms such as their institutional repository

- via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license - this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article.

Published journal article (JPA): A published journal article (PJA) is the definitive final record of published research that appears or will appear in the journal and embodies all value-adding publishing activities including peer review co-ordination, copy-editing, formatting, (if relevant) pagination and online enrichment.
Policies for sharing publishing journal articles differ for subscription and gold open access articles:
Subscription Articles: If you are an author, please share a link to your article rather than the full-text. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help your users to find, access, cite, and use the best available version.
Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.
If you are affiliated with a library that subscribes to ScienceDirect you have additional private sharing rights for others' research accessed under that agreement. This includes use for classroom teaching and internal training at the institution (including use in course packs and courseware programs), and inclusion of the article for grant funding purposes.
Gold Open Access Articles: May be shared according to the author-selected end-user license and should contain a CrossMark logo, the end user license, and a DOI link to the formal publication on ScienceDirect.
Please refer to Elsevier's posting policy for further information.
18. **For book authors** the following clauses are applicable in addition to the above:
Authors are permitted to place a brief summary of their work online only. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.
19. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for Proquest/UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission. Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

Elsevier Open Access Terms and Conditions
You can publish open access with Elsevier in hundreds of open access journals or in nearly

2000 established subscription journals that support open access publishing. Permitted third party re-use of these open access articles is defined by the author's choice of Creative Commons user license. See our open access license policy for more information.
Terms & Conditions applicable to all Open Access articles published with Elsevier:
Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation. If any changes have been made, such changes must be clearly indicated.
The author(s) must be appropriately credited and we ask that you include the end user license and a DOI link to the formal publication on ScienceDirect.
If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.
Additional Terms & Conditions applicable to each Creative Commons user license:
CC BY: The CC-BY license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article and to make commercial use of the Article (including reuse and/or resale of the Article by commercial entities), provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by/4.0.
CC BY NC SA: The CC BY-NC-SA license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article, provided this is not done for commercial purposes, and that the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. Further, any new works must be made available on the same conditions. The full details of the license are available at http://creativecommons.org/licenses/by-nc-sa/4.0.
CC BY NC ND: The CC BY-NC-ND license allows users to copy and distribute the Article, provided this is not done for commercial purposes and further does not permit distribution of the Article if it is changed or edited in any way, and provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, and that the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by-nc-nd/4.0.
Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.
Commercial reuse includes:

- Associating advertising with the full text of the Article
- Charging fees for document delivery or access
- Article aggregation
- Systematic distribution via e-mail lists or share buttons

Posting or linking by commercial companies for use by customers of those companies.

20. **Other Conditions**:

v1.8

Figure 1-7

WOLTERS KLUWER HEALTH, INC. LICENSE TERMS AND CONDITIONS

May 27, 2016

This Agreement between William Ngo ("You") and Wolters Kluwer Health, Inc. ("Wolters Kluwer Health, Inc.") consists of your license details and the terms and conditions provided by Wolters Kluwer Health, Inc. and Copyright Clearance Center.

License Number	3877190800591
License date	May 27, 2016
Licensed Content Publisher	Wolters Kluwer Health, Inc.
Licensed Content Publication	Cornea
Licensed Content Title	The Diurnal Secretory Characteristics of Individual Meibomian Glands.
Licensed Content Author	Blackie, Caroline; A OD, PhD; Korb, Donald
Licensed Content Date	Jan 1, 2010
Licensed Content Volume Number	29
Licensed Content Issue Number	1
Type of Use	Dissertation/Thesis
Requestor type	Individual
Portion	Figures/table/illustration
Number of figures/tables /illustrations	1
Figures/tables/illustrations used	Figure 1
Author of this Wolters Kluwer article	No
Title of your thesis / dissertation	Contemporary Diagnosis and Management of Dry Eye
Expected completion date	Aug 2016
Estimated size(pages)	200
Requestor Location	William Ngo School of Optometry & Vision Science University of Waterloo 200 University Avenue West Waterloo, ON N2L 3G1 Canada Attn: William Ngo
Billing Type	Invoice
Billing Address	William Ngo School of Optometry & Vision Science University of Waterloo 200 University Avenue West Waterloo, ON N2L 3G1

Canada
Attn: William Ngo

Total 0.00 CAD

Terms and Conditions

Terms and conditions Wolters Kluwer Health

1. **Transfer of License:** Wolters Kluwer hereby grants you a non-exclusive license to reproduce this material for this purpose, and for no other use, subject to the conditions herein
2. **Credit Line:** A credit line will be prominently placed, wherever the material is reused and include: the author(s), title of article, title of journal, volume number, issue number and inclusive pages.
   Where a journal is being published by a learned society, the details of that society must be included in the credit line.
   i. **for Open access journals:**The following statement needs to be added when reprinting the material in Open Access journals only: 'promotional and commercial use of the material in print, digital or mobile device format is prohibited without the permission from the publisher Wolters Kluwer Health. Please contact healthpermissions@wolterskluwer.com for further information
3. **Exceptions:** In case of ***Disease Colon Rectum, Plastic Reconstructive Surgery, The Green Journal, Critical care Medicine, Pediatric Critical Care Medicine, the American Heart Publications, the American Academy of Neurology*** the following guideline applies: no drug/ trade name or logo can be included in the same page as the material re-used.
4. **Translations:** When requesting a permission to translate a full text article, Wolters Kluwer/ Lippincott Williams & Wilkins request to receive the pdf of the translated document. This disclaimer should be added at all times:
   Wolters Kluwer Health and its Societies take no responsibility for the accuracy of the translation from the published English original and are not liable for any errors which may occur.
5. **Warranties** The requestor warrants that the material shall not be used in any manner which may be considered derogatory to the title, content, or authors of the material, or to Wolters Kluwer
6. **Indemnity:** You hereby indemnify and hold harmless Wolters Kluwer and their respective officers, directors, employees and agents, from and against any and all claims, costs, proceeding or demands arising out of your unauthorised use of the Licensed Material.
7. **Geographical Scope:** Permission granted is valid worldwide in the English language and the languages specified in your original request
8. Wolters Kluwer cannot supply the requestor with the original artwork or a "clean copy."
9. Permission is valid if the borrowed material is original to a Wolters Kluwer imprint (Lippincott-Raven Publishers, Williams &Wilkins, Lea & Febiger, Harwal, Rapid Science, Little Brown & Company, Harper & Row Medical, American Journal of Nursing Co, and Urban & Schwarzenberg)
10. **Termination of contract:** If you opt not to use the material requested above please notify RightsLink or Wolters Kluwer Health/ Lippincott Williams & Wilkins within 90 days of the original invoice date.
11. This permission does not apply to **images** that are credited to publications other than Wolters Kluwer journals. For images credited to non-Wolters Kluwer Health journal publications, you will need to obtain permission from the journal referenced in the figure or table legend or credit line before making any use of image(s) or table(s)
12. **Third party material:** Adaptations are protected by copyright, so if you would like to reuse material that we have adapted from another source, you will need not only our permission, but the permission of the rights holder of the original material. Similarly, if you want to reuse an adaptation of original LWW content that appears in another publishers work, you will need our permission and that of the next publisher. The adaptation should be credited as follows: Adapted with permission from Wolters Kluwer Health: Book author, title, year of publication or Journal name, article author, title, reference citation, year of publication.
13. **Altering or modifying material:** Please note that modification of text within figures or

full- text article is strictly forbidden.

14. Please note that articles in the **ahead-of–print stage** of publication can be cited and the content may be re-used by including the date of access and the unique DOI number. Any final changes in manuscripts will be made at the time of print publication and will be reflected in the final electronic issue. Disclaimer: Articles appearing in the Published Ahead-of–Print section have been peer-reviewed and accepted for publication in the relevant journal and posted online before print publication. Articles appearing as publish ahead–of-print may contain statements, opinions, and information that have errors in facts, figures, or interpretation. Accordingly, Lippincott Williams & Wilkins, the editors and authors and their respective employees are not responsible or liable for the use of any such inaccurate or misleading data, opinion or information contained in the articles in this section.
15. **Duration of the license:**
    i. Permission is granted for a one-time use only within 12 months from the date of this invoice. Rights herein do not apply to future reproductions, editors, revisions, or other derivative works. Once the 12- month term has expired, permission to renew must be submitted in writing.
    ii. For content reused in another journal or book, in print or electronic format, the license is one-time use and lasts for the 1st edition of a book or for the life of the edition in case of journals.
    iii. If your Permission Request is for use on a website (which is not a journal or a book), internet, intranet, or any publicly accessible site, you agree to remove the material from such site after 12 months or else renew your permission request.
16. **Contingent on payment:** *While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.*
17. **Waived permission fee:** If the permission fee for the requested use of our material has been waived in this instance, please be advised that your future requests for Wolters Kluwer materials may attract a fee on another occasion. Please always check with the Wolters Kluwer Permissions Team if in doubt healthpermissions@wolterskluwer.com

For Books only:

18. Permission is granted for a one time use only. Rights herein do not apply to future reproductions, editions, revisions, or other derivative works.

Service Description for Content Services

Subject to these terms of use, any terms set forth on the particular order, and payment of the applicable fee, you may make the following uses of the ordered materials:

• Content Rental: You may access and view a single electronic copy of the materials ordered for the time period designated at the time the order is placed. Access to the materials will be provided through a dedicated content viewer or other portal, and access will be discontinued upon expiration of the designated time period. An order for Content Rental does not include any rights to print, download, save, create additional copies, to distribute or to reuse in any way the full text or parts of the materials.

• Content Purchase: You may access and download a single electronic copy of the materials

ordered. Copies will be provided by email or by such other means as publisher may make available from time to time. An order for Content Purchase does not include any rights to create additional copies or to distribute copies of the materials.

The materials may be accessed and used only by the person who placed the Order or the person on whose behalf the order was placed and only in accordance with the terms included in the particular order.

SPECIAL CASES:

1. **For STM Signatories only, as agreed as part of the STM Guidelines**

Any permission granted for a particular edition will apply also to subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted illustrations or excerpts.

Please click here to view the STM guidelines.

Other Terms and Conditions:
v1.13

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Figure 3-1

Citation: Hwang HS, Shin JG, Lee BH, Eom TJ, Joo C-K (2013) In Vivo 3D Meibography of the Human Eyelid Using Real Time Imaging Fourier-Domain OCT. PLoS ONE 8(6): e67143. doi:10.1371/journal.pone.0067143

Editor: Yingfeng Zheng, Zhongshan Ophthalmic Center, China

Received: November 9, 2012; **Accepted:** May 13, 2013; **Published:** June 21, 2013

Funding: This work was partially supported by the Technology Innovation Program funded by the Ministry of Trade, Industry & Energy, Republic of Korea (10040121) and the Basic Science Research Program through the National Research Foundation of Korea (2010-0024058). Role of the funding source: development of high speed OCT for meibomian gland imaging.

Competing interests: The authors have declared that no competing interests exist.

Figure 3-2

WOLTERS KLUWER HEALTH, INC. LICENSE TERMS AND CONDITIONS

May 10, 2016

This Agreement between William Ngo ("You") and Wolters Kluwer Health, Inc. ("Wolters Kluwer Health, Inc.") consists of your license details and the terms and conditions provided by Wolters Kluwer Health, Inc. and Copyright Clearance Center.

License Number	3865510392508
License date	May 10, 2016
Licensed Content Publisher	Wolters Kluwer Health, Inc.
Licensed Content Publication	Cornea
Licensed Content Title	Novel Noncontact Meibography With Anterior Segment Optical Coherence Tomography: Hosik Meibography.
Licensed Content Author	Hwang, Ho; Park, Chang; Joo, Choun-Ki; MD, PhD
Licensed Content Date	Jan 1, 2013
Licensed Content Volume Number	32
Licensed Content Issue Number	1
Type of Use	Dissertation/Thesis
Requestor type	Individual
Portion	Figures/table/illustration
Number of figures/tables /illustrations	1
Figures/tables/illustrations used	3
Author of this Wolters Kluwer article	No
Title of your thesis / dissertation	Contemporary Diagnosis and Management of Dry Eye
Expected completion date	Aug 2016
Estimated size(pages)	200
Requestor Location	William Ngo School of Optometry & Vision Science University of Waterloo 200 University Avenue West Waterloo, ON N2L 3G1 Canada Attn: William Ngo
Billing Type	Invoice
Billing Address	William Ngo School of Optometry & Vision Science University of Waterloo 200 University Avenue West Waterloo, ON N2L 3G1

Canada
Attn: William Ngo

Total 0.00 CAD

Terms and Conditions

Terms and conditions Wolters Kluwer Health

1. **Transfer of License:** Wolters Kluwer hereby grants you a non-exclusive license to reproduce this material for this purpose, and for no other use, subject to the conditions herein
2. **Credit Line:** A credit line will be prominently placed, wherever the material is reused and include: the author(s), title of article, title of journal, volume number, issue number and inclusive pages.
   Where a journal is being published by a learned society, the details of that society must be included in the credit line.
   i. **for Open access journals:**The following statement needs to be added when reprinting the material in Open Access journals only: 'promotional and commercial use of the material in print, digital or mobile device format is prohibited without the permission from the publisher Wolters Kluwer Health. Please contact healthpermissions@wolterskluwer.com for further information
3. **Exceptions:** In case of ***Disease Colon Rectum, Plastic Reconstructive Surgery, The Green Journal, Critical care Medicine, Pediatric Critical Care Medicine, the American Heart Publications, the American Academy of Neurology*** the following guideline applies: no drug/ trade name or logo can be included in the same page as the material re-used.
4. **Translations:** When requesting a permission to translate a full text article, Wolters Kluwer/ Lippincott Williams & Wilkins request to receive the pdf of the translated document. This disclaimer should be added at all times:
   Wolters Kluwer Health and its Societies take no responsibility for the accuracy of the translation from the published English original and are not liable for any errors which may occur.
5. **Warranties** The requestor warrants that the material shall not be used in any manner which may be considered derogatory to the title, content, or authors of the material, or to Wolters Kluwer
6. **Indemnity:** You hereby indemnify and hold harmless Wolters Kluwer and their respective officers, directors, employees and agents, from and against any and all claims, costs, proceeding or demands arising out of your unauthorised use of the Licensed Material.
7. **Geographical Scope:** Permission granted is valid worldwide in the English language and the languages specified in your original request
8. Wolters Kluwer cannot supply the requestor with the original artwork or a "clean copy."
9. Permission is valid if the borrowed material is original to a Wolters Kluwer imprint (Lippincott-Raven Publishers, Williams &Wilkins, Lea & Febiger, Harwal, Rapid Science, Little Brown & Company, Harper & Row Medical, American Journal of Nursing Co, and Urban & Schwarzenberg)
10. **Termination of contract:** If you opt not to use the material requested above please notify RightsLink or Wolters Kluwer Health/ Lippincott Williams & Wilkins within 90 days of the original invoice date.
11. This permission does not apply to **images** that are credited to publications other than Wolters Kluwer journals. For images credited to non-Wolters Kluwer Health journal publications, you will need to obtain permission from the journal referenced in the figure or table legend or credit line before making any use of image(s) or table(s)
12. **Third party material:** Adaptations are protected by copyright, so if you would like to reuse material that we have adapted from another source, you will need not only our permission, but the permission of the rights holder of the original material. Similarly, if you want to reuse an adaptation of original LWW content that appears in another publishers work, you will need our permission and that of the next publisher. The adaptation should be credited as follows: Adapted with permission from Wolters Kluwer Health: Book author, title, year of publication or Journal name, article author, title, reference citation, year of publication.
13. **Altering or modifying material:** Please note that modification of text within figures or

full- text article is strictly forbidden.

14. Please note that articles in the **ahead-of–print stage** of publication can be cited and the content may be re-used by including the date of access and the unique DOI number. Any final changes in manuscripts will be made at the time of print publication and will be reflected in the final electronic issue. Disclaimer: Articles appearing in the Published Ahead-of–Print section have been peer-reviewed and accepted for publication in the relevant journal and posted online before print publication. Articles appearing as publish ahead–of-print may contain statements, opinions, and information that have errors in facts, figures, or interpretation. Accordingly, Lippincott Williams & Wilkins, the editors and authors and their respective employees are not responsible or liable for the use of any such inaccurate or misleading data, opinion or information contained in the articles in this section.
15. **Duration of the license:**
    i. Permission is granted for a one-time use only within 12 months from the date of this invoice. Rights herein do not apply to future reproductions, editors, revisions, or other derivative works. Once the 12- month term has expired, permission to renew must be submitted in writing.
    ii. For content reused in another journal or book, in print or electronic format, the license is one-time use and lasts for the 1st edition of a book or for the life of the edition in case of journals.
    iii. If your Permission Request is for use on a website (which is not a journal or a book), internet, intranet, or any publicly accessible site, you agree to remove the material from such site after 12 months or else renew your permission request.
16. **Contingent on payment:** *While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.*
17. **Waived permission fee:** If the permission fee for the requested use of our material has been waived in this instance, please be advised that your future requests for Wolters Kluwer materials may attract a fee on another occasion. Please always check with the Wolters Kluwer Permissions Team if in doubt healthpermissions@wolterskluwer.com

For Books only:

18. Permission is granted for a one time use only. Rights herein do not apply to future reproductions, editions, revisions, or other derivative works.

Service Description for Content Services

Subject to these terms of use, any terms set forth on the particular order, and payment of the applicable fee, you may make the following uses of the ordered materials:

• Content Rental: You may access and view a single electronic copy of the materials ordered for the time period designated at the time the order is placed. Access to the materials will be provided through a dedicated content viewer or other portal, and access will be discontinued upon expiration of the designated time period. An order for Content Rental does not include any rights to print, download, save, create additional copies, to distribute or to reuse in any way the full text or parts of the materials.

• Content Purchase: You may access and download a single electronic copy of the materials

ordered. Copies will be provided by email or by such other means as publisher may make available from time to time. An order for Content Purchase does not include any rights to create additional copies or to distribute copies of the materials.

The materials may be accessed and used only by the person who placed the Order or the person on whose behalf the order was placed and only in accordance with the terms included in the particular order.

SPECIAL CASES:

1. **For STM Signatories only, as agreed as part of the STM Guidelines**

Any permission granted for a particular edition will apply also to subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted illustrations or excerpts.

Please click here to view the STM guidelines.

Other Terms and Conditions:
v1.13

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Figure 3-3

BioMed Research International
Volume 2016 (2016), Article ID 7432131, 8 pages
http://dx.doi.org/10.1155/2016/7432131

Review Article

In Vivo Laser Scanning Confocal Microscopy of Human Meibomian Glands in Aging and Ocular Surface Diseases

Vincenzo Fasanella,[1] Luca Agnifili,[1] Rodolfo Mastropasqua,[2] Lorenza Brescia,[1] Federico Di Staso,[3] Marco Ciancaglini,[3] and Leonardo Mastropasqua[1]

[1]Ophthalmology Clinic, Department of Medicine and Aging Science, "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy
[2]Ophthalmology Unit, Department of Neurological, Neuropsychological, Morphological and Movement Sciences, University of Verona, 37126 Verona, Italy
[3]Ophthalmic Clinic, Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy

Received 13 November 2015; Revised 8 February 2016; Accepted 17 February 2016

Academic Editor: Dipika V. Patel

Figure 3-11

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

May 11, 2016

This Agreement between William Ngo ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	3865970022042
License date	May 11, 2016
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Journal of Anatomy
Licensed Content Title	The lid wiper and muco-cutaneous junction anatomy of the human eyelid margins: an in vivo confocal and histological study
Licensed Content Author	Erich Knop,Nadja Knop,Andrey Zhivov,Robert Kraak,Donald R. Korb,Caroline Blackie,Jack V. Greiner,Rudolf Guthoff
Licensed Content Date	Mar 18, 2011
Pages	13
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	1
Original Wiley figure/table number(s)	Figure 2
Will you be translating?	No
Title of your thesis / dissertation	Contemporary Diagnosis and Management of Dry Eye
Expected completion date	Aug 2016
Expected size (number of pages)	200
Requestor Location	William Ngo School of Optometry & Vision Science University of Waterloo 200 University Avenue West Waterloo, ON N2L 3G1 Canada Attn: William Ngo
Billing Type	Invoice
Billing Address	William Ngo School of Optometry & Vision Science University of Waterloo 200 University Avenue West Waterloo, ON N2L 3G1 Canada Attn: William Ngo

Total 0.00 CAD

Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone

basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or

certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online Library http://olabout.wiley.com/WileyCDA/Section/id-410895.html

Other Terms and Conditions:

v1.10 Last updated September 2015

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Figure 3-12

ELSEVIER LICENSE
TERMS AND CONDITIONS

May 11, 2016

This is a License Agreement between William Ngo ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited The Boulevard,Langford Lane Kidlington,Oxford,OX5 1GB,UK
Registered Company Number	1982084
Customer name	William Ngo
Customer address	School of Optometry & Vision Science Waterloo, ON N2L 3G1
License number	3865950653226
License date	May 11, 2016
Licensed content publisher	Elsevier
Licensed content publication	Journal of the American Academy of Dermatology
Licensed content title	New insights into nevogenesis: In vivo characterization and follow-up of melanocytic nevi by reflectance confocal microscopy
Licensed content author	Giovanni Pellacani,Alon Scope,Barbara Ferrari,Gaia Pupelli,Sara Bassoli,Caterina Longo,Anna Maria Cesinaro,Giuseppe Argenziano,Rainer Hofmann-Wellenhof,Josep Malvehy,Ashfaq A. Marghoob,Susana Puig,Stefania Seidenari,H. Peter Soyer,Iris Zalaudek
Licensed content date	December 2009
Licensed content volume number	61
Licensed content issue number	6
Number of pages	13
Start Page	1001
End Page	1013
Type of Use	reuse in a thesis/dissertation
Intended publisher of new work	other
Portion	figures/tables/illustrations
Number of figures/tables /illustrations	1
Format	both print and electronic

Are you the author of this Elsevier article?	No
Will you be translating?	No
Original figure numbers	figure 1
Title of your thesis/dissertation	Contemporary Diagnosis and Management of Dry Eye
Expected completion date	Aug 2016
Estimated size (number of pages)	200
Elsevier VAT number	GB 494 6272 12
Permissions price	0.00 CAD
VAT/Local Sales Tax	0.00 CAD / 0.00 GBP
Total	0.00 CAD
Terms and Conditions	

licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator

must perform all translations and reproduce the content word for word preserving the integrity of the article.
16. **Posting licensed content on any Website**: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.
Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com . All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.
17. **For journal authors:** the following clauses are applicable in addition to the above:
Preprints:
A preprint is an author's own write-up of research results and analysis, it has not been peer-reviewed, nor has it had any other value added to it by a publisher (such as formatting, copyright, technical enhancement etc.).
Authors can share their preprints anywhere at any time. Preprints should not be added to or enhanced in any way in order to appear more like, or to substitute for, the final versions of articles however authors can update their preprints on arXiv or RePEc with their Accepted Author Manuscript (see below).
If accepted for publication, we encourage authors to link from the preprint to their formal publication via its DOI. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help users to find, access, cite and use the best available version. Please note that Cell Press, The Lancet and some society-owned have different preprint policies. Information on these policies is available on the journal homepage.
Accepted Author Manuscripts: An accepted author manuscript is the manuscript of an article that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and editor-author communications.
Authors can share their accepted author manuscript:

- immediately
    - via their non-commercial person homepage or blog
    - by updating a preprint in arXiv or RePEc with the accepted manuscript
    - via their research institute or institutional repository for internal institutional uses or as part of an invitation-only research collaboration work-group
    - directly by providing copies to their students or to research collaborators for their personal use
    - for private scholarly sharing as part of an invitation-only work group on commercial sites with which Elsevier has an agreement

- after the embargo period
  - via non-commercial hosting platforms such as their institutional repository
  - via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license - this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article.

Published journal article (JPA): A published journal article (PJA) is the definitive final record of published research that appears or will appear in the journal and embodies all value-adding publishing activities including peer review co-ordination, copy-editing, formatting, (if relevant) pagination and online enrichment.
Policies for sharing publishing journal articles differ for subscription and gold open access articles:
Subscription Articles: If you are an author, please share a link to your article rather than the full-text. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help your users to find, access, cite, and use the best available version.
Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.
If you are affiliated with a library that subscribes to ScienceDirect you have additional private sharing rights for others' research accessed under that agreement. This includes use for classroom teaching and internal training at the institution (including use in course packs and courseware programs), and inclusion of the article for grant funding purposes.
Gold Open Access Articles: May be shared according to the author-selected end-user license and should contain a CrossMark logo, the end user license, and a DOI link to the formal publication on ScienceDirect.
Please refer to Elsevier's posting policy for further information.
18. **For book authors** the following clauses are applicable in addition to the above:
Authors are permitted to place a brief summary of their work online only. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.
19. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for Proquest/UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission. Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

Elsevier Open Access Terms and Conditions

You can publish open access with Elsevier in hundreds of open access journals or in nearly 2000 established subscription journals that support open access publishing. Permitted third party re-use of these open access articles is defined by the author's choice of Creative Commons user license. See our open access license policy for more information.

Terms & Conditions applicable to all Open Access articles published with Elsevier:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation. If any changes have been made, such changes must be clearly indicated.

The author(s) must be appropriately credited and we ask that you include the end user license and a DOI link to the formal publication on ScienceDirect.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: The CC-BY license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article and to make commercial use of the Article (including reuse and/or resale of the Article by commercial entities), provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by/4.0.

CC BY NC SA: The CC BY-NC-SA license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article, provided this is not done for commercial purposes, and that the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. Further, any new works must be made available on the same conditions. The full details of the license are available at http://creativecommons.org/licenses/by-nc-sa/4.0.

CC BY NC ND: The CC BY-NC-ND license allows users to copy and distribute the Article, provided this is not done for commercial purposes and further does not permit distribution of the Article if it is changed or edited in any way, and provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, and that the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by-nc-nd/4.0.

Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

- Associating advertising with the full text of the Article
- Charging fees for document delivery or access
- Article aggregation
- Systematic distribution via e-mail lists or share buttons

Posting or linking by commercial companies for use by customers of those companies.

20. **Other Conditions**:

v1.8

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Chapter 4 - Repeatability of Grading Meibomian Gland Dropout Using Two Infrared Systems

Reply Reply All Forward

Fwd: RE: question re published article and thesis

Lyndon Jones [lwjones@connect.uwaterloo.ca]

To: William Ngo; Sruthi Srinivasan

August 11, 2016 9:30 AM

You replied on 11/08/2016 10:48 AM.

FYI

-------- Forwarded Message --------
Subject:RE: question re published article and thesis
Date: Thu, 11 Aug 2016 13:26:22 +0000
From:OVS <ovs@osu.edu>
To: Lyndon Jones <lwjones@uwaterloo.ca>

Yes.

K

****
Optometry and Vision Science
Kurt A. Zadnik, Managing Editor
The Ohio State University, College of Optometry
338 West 10th Avenue
Columbus, OH 43210
Tel: (614) 292-4942; Fax: (614) 292-4949;
e-mail: ovs@osu.edu
http://ovs.edmgr.com
****

From: Lyndon Jones [mailto:lwjones@uwaterloo.ca]
Sent: Wednesday, August 10, 2016 5:04 PM
To: OVS <ovs@osu.edu>
Cc: William Ngo <william.ngo@uwaterloo.ca>; Sruthi Srinivasan <s2srinivasan@uwaterloo.ca>
Subject: question re published article and thesis

Hi Kurt,
William Ngo had his PhD thesis exam today - which he passed. Two of the chapters within the thesis were:
1. Ngo W, Srinivasan S, Schulze M, Jones L. Repeatability of grading meibomian gland dropout using two infrared systems. Optometry and vision science : official publication of the American Academy of Optometry 2014; 91(6):658-67.
2. Ngo W, Caffery B, Srinivasan S, Jones LW. Effect of lid debridement-scaling in Sjogren Syndrome dry eye. Optometry and vision science : official publication of the American Academy of Optometry 2015; 92(9):e316-20.
The external examiner has requested some changes made to these chapters in terms of expansion of the methods and a small amount of extra data analysis. Thus, the chapter will no longer be identical to that in OVS. Will received permission to use these papers in his thesis and acknowledges that in the thesis (that those chapters were published

Chapter 7 - The Effect of Lid Debridement-Scaling in Sjögren's Syndrome Dry Eye

Reply Reply All Forward

Fwd: RE: question re published article and thesis

Lyndon Jones [lwjones@connect.uwaterloo.ca]

To: William Ngo; Sruthi Srinivasan

August 11, 2016 9:30 AM

You replied on 11/08/2016 10:48 AM.

FYI

-------- Forwarded Message --------
Subject:RE: question re published article and thesis
Date: Thu, 11 Aug 2016 13:26:22 +0000
From:OVS <ovs@osu.edu>
To: Lyndon Jones <lwjones@uwaterloo.ca>

Yes.

K

****
Optometry and Vision Science
Kurt A. Zadnik, Managing Editor
The Ohio State University, College of Optometry
338 West 10th Avenue
Columbus, OH 43210
Tel: (614) 292-4942; Fax: (614) 292-4949;
e-mail: ovs@osu.edu
http://ovs.edmgr.com
****

From: Lyndon Jones [mailto:lwjones@uwaterloo.ca]
Sent: Wednesday, August 10, 2016 5:04 PM
To: OVS <ovs@osu.edu>
Cc: William Ngo <william.ngo@uwaterloo.ca>; Sruthi Srinivasan <s2srinivasan@uwaterloo.ca>
Subject: question re published article and thesis

Hi Kurt,
William Ngo had his PhD thesis exam today - which he passed. Two of the chapters within the thesis were:
1. Ngo W, Srinivasan S, Schulze M, Jones L. Repeatability of grading meibomian gland dropout using two infrared systems. Optometry and vision science : official publication of the American Academy of Optometry 2014; 91(6):658-67.
2. Ngo W, Caffery B, Srinivasan S, Jones LW. Effect of lid debridement-scaling in Sjogren Syndrome dry eye. Optometry and vision science : official publication of the American Academy of Optometry 2015; 92(9):e316-20.
The external examiner has requested some changes made to these chapters in terms of expansion of the methods and a small amount of extra data analysis. Thus, the chapter will no longer be identical to that in OVS. Will received permission to use these papers in his thesis and acknowledges that in the thesis (that those chapters were published

References

References from Literature Review

1. Smith JA, Albeitz J, Begley C, et al. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):93-107.

2. Schein OD, Hochberg MC, Munoz B, et al. Dry eye and dry mouth in the elderly: a population-based assessment. *Arch Intern Med.* Jun 28 1999;159(12):1359-1363.

3. Schein OD, Munoz B, Tielsch JM, Bandeen-Roche K, West S. Prevalence of dry eye among the elderly. *Am J Ophthalmol.* Dec 1997;124(6):723-728.

4. Munoz B, West SK, Rubin GS, et al. Causes of blindness and visual impairment in a population of older Americans: The Salisbury Eye Evaluation Study. *Arch Ophthalmol.* Jun 2000;118(6):819-825.

5. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. *Arch Ophthalmol.* Sep 2000;118(9):1264-1268.

6. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol.* Aug 2003;136(2):318-326.

7. Christen WG, Manson JE, Glynn RJ, et al. Low-dose aspirin and risk of cataract and subtypes in a randomized trial of U.S. physicians. *Ophthalmic Epidemiol.* Sep 1998;5(3):133-142.

8. Christen WG, Gaziano JM, Hennekens CH. Design of Physicians' Health Study II--a randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. *Ann Epidemiol.* Feb 2000;10(2):125-134.

9. Schaumberg DA, Dana R, Buring JE, Sullivan DA. Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. *Arch Ophthalmol.* Jun 2009;127(6):763-768.

10. Chia EM, Mitchell P, Rochtchina E, Lee AJ, Maroun R, Wang JJ. Prevalence and associations of dry eye syndrome in an older population: the Blue Mountains Eye Study. *Clin Experiment Ophthalmol.* Jun 2003;31(3):229-232.

11. McCarty CA, Bansal AK, Livingston PM, Stanislavsky YL, Taylor HR. The epidemiology of dry eye in Melbourne, Australia. *Ophthalmology.* Jun 1998;105(6):1114-1119.

12. Lin PY, Tsai SY, Cheng CY, Liu JH, Chou P, Hsu WM. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology.* Jun 2003;110(6):1096-1101.

13. Lee AJ, Lee J, Saw SM, et al. Prevalence and risk factors associated with dry eye symptoms: a population based study in Indonesia. *Br J Ophthalmol.* Dec 2002;86(12):1347-1351.

14. Bakkar MM, Shihadeh WA, Haddad MF, Khader YS. Epidemiology of symptoms of dry eye disease (DED) in Jordan: A cross-sectional non-clinical population-based study. *Cont Lens Anterior Eye.* Jan 29 2016.

15. Uchino M, Nishiwaki Y, Michikawa T, et al. Prevalence and Risk Factors of Dry Eye Disease in Japan: Koumi Study. *Ophthalmology.* Dec 2011;118(12):2361-2367.

16. Uchino M, Schaumberg DA, Dogru M, et al. Prevalence of dry eye disease among Japanese visual display terminal users. *Ophthalmology.* Nov 2008;115(11):1982-1988.

17. Ahn JM, Lee SH, Rim TH, et al. Prevalence of and risk factors associated with dry eye: the Korea National Health and Nutrition Examination Survey 2010-2011. *Am J Ophthalmol.* Dec 2014;158(6):1205-1214 e1207.

18. Um SB, Kim NH, Lee HK, Song JS, Kim HC. Spatial epidemiology of dry eye disease: findings from South Korea. *Int J Health Geogr.* 2014;13:31.

19. Hashemi H, Khabazkhoob M, Kheirkhah A, et al. Prevalence of dry eye syndrome in an adult population. *Clin Experiment Ophthalmol.* Apr 2014;42(3):242-248.

20. Liu NN, Liu L, Li J, Sun YZ. Prevalence of and risk factors for dry eye symptom in mainland china: a systematic review and meta-analysis. *J Ophthalmol.* 2014;2014:748654.

21. Sharma A, Hindman HB. Aging: a predisposition to dry eyes. *J Ophthalmol.* 2014;2014:781683.

22. Vehof J, Kozareva D, Hysi PG, Hammond CJ. Prevalence and risk factors of dry eye disease in a British female cohort. *Br J Ophthalmol.* Dec 2014;98(12):1712-1717.

23. Akpek EK, Smith RA. Overview of age-related ocular conditions. *Am J Manag Care.* May 2013;19(5 Suppl):S67-75.

24. Uchino Y, Kawakita T, Miyazawa M, et al. Oxidative stress induced inflammation initiates functional decline of tear production. *PLoS One.* 2012;7(10):e45805.

25. Uchino Y, Kawakita T, Ishii T, Ishii N, Tsubota K. A new mouse model of dry eye disease: oxidative stress affects functional decline in the lacrimal gland. *Cornea.* Nov 2012;31 Suppl 1:S63-67.

26. Kojima T, Wakamatsu TH, Dogru M, et al. Age-related dysfunction of the lacrimal gland and oxidative stress: evidence from the Cu,Zn-superoxide dismutase-1 (Sod1) knockout mice. *Am J Pathol.* May 2012;180(5):1879-1896.

27. Batista TM, Tomiyoshi LM, Dias AC, et al. Age-dependent changes in rat lacrimal gland anti-oxidant and vesicular related protein expression profiles. *Mol Vis.* 2012;18:194-202.

28. Rocha EM, Alves M, Rios JD, Dartt DA. The aging lacrimal gland: changes in structure and function. *Ocul Surf.* Oct 2008;6(4):162-174.

29. Modulo CM, Jorge AG, Dias AC, et al. Influence of insulin treatment on the lacrimal gland and ocular surface of diabetic rats. *Endocrine.* Aug 2009;36(1):161-168.

30. Jorge AG, Modulo CM, Dias AC, et al. Aspirin prevents diabetic oxidative changes in rat lacrimal gland structure and function. *Endocrine.* Apr 2009;35(2):189-197.

31. Rocha EM, Carvalho CR, Saad MJ, Velloso LA. The influence of ageing on the insulin signalling system in rat lacrimal and salivary glands. *Acta Ophthalmol Scand.* Dec 2003;81(6):639-645.

32. Paulsen AJ, Cruickshanks KJ, Fischer ME, et al. Dry eye in the beaver dam offspring study: prevalence, risk factors, and health-related quality of life. *Am J Ophthalmol.* Apr 2014;157(4):799-806.

33. Truong S, Cole N, Stapleton F, Golebiowski B. Sex hormones and the dry eye. *Clin Exp Optom.* Jul 2014;97(4):324-336.

34. Zouboulis CC, Makrantonaki E. Hormonal therapy of intrinsic aging. *Rejuvenation Res.* Jun 2012;15(3):302-312.

35. Erdem U, Ozdegirmenci O, Sobaci E, Sobaci G, Goktolga U, Dagli S. Dry eye in post-menopausal women using hormone replacement therapy. *Maturitas.* Mar 20 2007;56(3):257-262.

36. Yang WJ, Yang YN, Cao J, et al. Risk Factors for Dry Eye Syndrome: A Retrospective Case-Control Study. *Optom Vis Sci.* Sep 2015;92(9):e199-205.

37. Sjogren H. Some problems concerning keratoconjunctivitis sicca and the sicca-syndrome. *Acta Ophthalmol (Copenh).* 1951;29(1):33-47.

38. Read RW. Clinical mini-review: systemic lupus erythematosus and the eye. *Ocul Immunol Inflamm.* Jun 2004;12(2):87-99.

39. Roh HC, Lee JK, Kim M, et al. Systemic Comorbidities of Dry Eye Syndrome: The Korean National Health and Nutrition Examination Survey V, 2010 to 2012. *Cornea.* Feb 2016;35(2):187-192.

40. Scott G, Balsiger H, Kluckman M, Fan J, Gest T. Patterns of innervation of the lacrimal gland with clinical application. *Clin Anat.* Nov 2014;27(8):1174-1177.

41. Kocer E, Kocer A, Ozsutcu M, Dursun AE, Krpnar I. Dry Eye Related to Commonly Used New Antidepressants. *J Clin Psychopharmacol.* Aug 2015;35(4):411-413.

42. Wong J, Lan W, Ong LM, Tong L. Non-hormonal systemic medications and dry eye. *Ocul Surf.* Oct 2011;9(4):212-226.

43. Ozen Tunay Z, Ozdemir O, Erginturk Acar D, Cavkaytar S, Ersoy E. Dry eye findings worsen with anticholinergic therapy in patients with urge incontinence. *Int Urogynecol J.* Dec 7 2015.

44. Nelson AM, Gilliland KL, Cong Z, Thiboutot DM. 13-cis Retinoic acid induces apoptosis and cell cycle arrest in human SEB-1 sebocytes. *J Invest Dermatol.* Oct 2006;126(10):2178-2189.

45. Layton A. The use of isotretinoin in acne. *Dermato-endocrinology.* May-Jun 2009;1(3):162-169.

46. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1938-1978.

47. Moy A, McNamara NA, Lin MC. Effects of Isotretinoin on Meibomian Glands. *Optom Vis Sci.* Sep 2015;92(9):925-930.

48. Ding J, Kam WR, Dieckow J, Sullivan DA. The influence of 13-cis retinoic acid on human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci.* Jun 2013;54(6):4341-4350.

49. Kremer I, Gaton DD, David M, Gaton E, Shapiro A. Toxic effects of systemic retinoids on meibomian glands. *Ophthalmic Res.* 1994;26(2):124-128.

50. Mathers WD, Shields WJ, Sachdev MS, Petroll WM, Jester JV. Meibomian gland morphology and tear osmolarity: changes with Accutane therapy. *Cornea.* Jul 1991;10(4):286-290.

51. Lambert RW, Smith RE. Effects of 13-cis-retinoic acid on the hamster meibomian gland. *J Invest Dermatol.* Mar 1989;92(3):321-325.

52. Azuma M, Yabuta C, Fraunfelder FW, Shearer TR. Dry eye in LASIK patients. *BMC Res Notes.* 2014;7:420.

53. Shtein RM. Post-LASIK dry eye. *Expert Rev Ophthalmol.* Oct 2011;6(5):575-582.

54. Benitez-del-Castillo JM, del Rio T, Iradier T, Hernandez JL, Castillo A, Garcia-Sanchez J. Decrease in tear secretion and corneal sensitivity after laser in situ keratomileusis. *Cornea.* Jan 2001;20(1):30-32.

55. Perez-Santonja JJ, Sakla HF, Cardona C, Chipont E, Alio JL. Corneal sensitivity after photorefractive keratectomy and laser in situ keratomileusis for low myopia. *Am J Ophthalmol.* May 1999;127(5):497-504.

56. Shin SY, Lee YJ. Conjunctival changes induced by LASIK suction ring in a rabbit model. *Ophthalmic Res.* 2006;38(6):343-349.

57. Rodriguez AE, Rodriguez-Prats JL, Hamdi IM, Galal A, Awadalla M, Alio JL. Comparison of goblet cell density after femtosecond laser and mechanical microkeratome in LASIK. *Invest Ophthalmol Vis Sci.* Jun 2007;48(6):2570-2575.

58. Ji H, Chen A, Zhang W, Gu H, Zhang Z, Fu J. Dynamic changes of tear fluid matrix metralloproteinase-9 within 1 year after laser in situ keratomileusis. *Nan Fang Yi Ke Da Xue Xue Bao.* Jul 2014;34(8):1079-1082.

59. Resan M, Stanojevic I, Petkovic A, Pajic B, Vojvodic D. Levels of interleukin-6 in tears before and after excimer laser treatment. *Vojnosanit Pregl.* Apr 2015;72(4):350-355.

60. Battat L, Macri A, Dursun D, Pflugfelder SC. Effects of laser in situ keratomileusis on tear production, clearance, and the ocular surface. *Ophthalmology.* Jul 2001;108(7):1230-1235.

61. Bower KS, Sia RK, Ryan DS, Mines MJ, Dartt DA. Chronic dry eye in photorefractive keratectomy and laser in situ keratomileusis: Manifestations, incidence, and predictive factors. *J Cataract Refract Surg.* Dec 2015;41(12):2624-2634.

62. Yu Y, Hua H, Wu M, Yu W, Lai K, Yao K. Evaluation of dry eye after femtosecond laser-assisted cataract surgery. *J Cataract Refract Surg.* Dec 2015;41(12):2614-2623.

63. Sutu C, Fukuoka H, Afshari NA. Mechanisms and management of dry eye in cataract surgery patients. *Curr Opin Ophthalmol.* Nov 13 2015.

64. Lopez-Miguel A, Teson M, Martin-Montanez V, et al. Clinical and Molecular Inflammatory Response in Sjogren Syndrome-Associated Dry Eye Patients Under Desiccating Stress. *Am J Ophthalmol.* Jan 2016;161:133-141 e132.

65. Teson M, Gonzalez-Garcia MJ, Lopez-Miguel A, et al. Influence of a controlled environment simulating an in-flight airplane cabin on dry eye disease. *Invest Ophthalmol Vis Sci.* Mar 2013;54(3):2093-2099.

66. Suhalim JL, Parfitt GJ, Xie Y, et al. Effect of desiccating stress on mouse meibomian gland function. *Ocul Surf.* Jan 2014;12(1):59-68.

67. Argiles M, Cardona G, Perez-Cabre E, Rodriguez M. Blink Rate and Incomplete Blinks in Six Different Controlled Hard-Copy and Electronic Reading Conditions. *Invest Ophthalmol Vis Sci.* Oct 2015;56(11):6679-6685.

68. Kojima T, Ibrahim OM, Wakamatsu T, et al. The impact of contact lens wear and visual display terminal work on ocular surface and tear functions in office workers. *Am J Ophthalmol.* Dec 2011;152(6):933-940 e932.

69. Teson M, Lopez-Miguel A, Neves H, Calonge M, Gonzalez-Garcia MJ, Gonzalez-Meijome JM. Influence of Climate on Clinical Diagnostic Dry Eye Tests: Pilot Study. *Optom Vis Sci.* Sep 2015;92(9):e284-289.

70. Mann A, Tighe B. Contact lens interactions with the tear film. *Exp Eye Res.* Dec 2013;117:88-98.

71. Suwala M, Glasier MA, Subbaraman LN, Jones L. Quantity and conformation of lysozyme deposited on conventional and silicone hydrogel contact lens materials using an in vitro model. *Eye Contact Lens.* May 2007;33(3):138-143.

72. Ng A, Heynen M, Luensmann D, Subbaraman LN, Jones L. Impact of tear film components on the conformational state of lysozyme deposited on contact lenses. *J Biomed Mater Res B Appl Biomater.* Oct 2013;101(7):1172-1181.

73. Panaser A, Tighe BJ. Evidence of lipid degradation during overnight contact lens wear: gas chromatography mass spectrometry as the diagnostic tool. *Invest Ophthalmol Vis Sci.* Mar 2014;55(3):1797-1804.

74. Skotnitsky CC, Naduvilath TJ, Sweeney DF, Sankaridurg PR. Two presentations of contact lens-induced papillary conjunctivitis (CLPC) in hydrogel lens wear: local and general. *Optom Vis Sci.* Jan 2006;83(1):27-36.

75. Dumbleton K, Woods CA, Jones LW, Fonn D. The impact of contemporary contact lenses on contact lens discontinuation. *Eye Contact Lens.* Jan 2013;39(1):93-99.

76. Sindt CW, Longmuir RA. Contact lens strategies for the patient with dry eye. *Ocul Surf.* Oct 2007;5(4):294-307.

77. Epstein SP, Ahdoot M, Marcus E, Asbell PA. Comparative toxicity of preservatives on immortalized corneal and conjunctival epithelial cells. *J Ocul Pharmacol Ther.* Apr 2009;25(2):113-119.

78. Noecker R. Effects of common ophthalmic preservatives on ocular health. *Adv Ther.* Sep-Oct 2001;18(5):205-215.

79. Ryan G, Jr., Fain JM, Lovelace C, Gelotte KM. Effectiveness of ophthalmic solution preservatives: a comparison of latanoprost with 0.02% benzalkonium chloride and travoprost with the sofZia preservative system. *BMC Ophthalmol.* 2011;11:8.

80. Chen W, Dong N, Huang C, et al. Corneal alterations induced by topical application of commercial latanoprost, travoprost and bimatoprost in rabbit. *PLoS One.* 2014;9(3):e89205.

81. Kahook MY, Noecker R. Quantitative analysis of conjunctival goblet cells after chronic application of topical drops. *Adv Ther.* Aug 2008;25(8):743-751.

82. Tomic M, Kastelan S, Soldo KM, Salopek-Rabatic J. Influence of BAK-preserved prostaglandin analog treatment on the ocular surface health in patients with newly diagnosed primary open-angle glaucoma. *Biomed Res Int.* 2013;2013:603782.

83. Epstein SP, Chen D, Asbell PA. Evaluation of biomarkers of inflammation in response to benzalkonium chloride on corneal and conjunctival epithelial cells. *J Ocul Pharmacol Ther.* Oct 2009;25(5):415-424.

84. Saade CE, Lari HB, Berezina TL, Fechtner RD, Khouri AS. Topical glaucoma therapy and ocular surface disease: a prospective, controlled cohort study. *Can J Ophthalmol.* Apr 2015;50(2):132-136.

85. Aihara M, Ikeda Y, Mizoue S, Arakaki Y, Kita N, Kobayashi S. Effect of Switching to Travoprost Preserved With SofZia in Glaucoma Patients With Chronic Superficial Punctate Keratitis While Receiving BAK-preserved Latanoprost. *J Glaucoma.* May 2 2015.

86. Kim JH, Kim EJ, Kim YH, et al. In Vivo Effects of Preservative-free and Preserved Prostaglandin Analogs: Mouse Ocular Surface Study. *Korean J Ophthalmol.* Aug 2015;29(4):270-279.

87. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):75-92.

88. Peri Y, Agmon-Levin N, Theodor E, Shoenfeld Y. Sjogren's syndrome, the old and the new. *Best Pract Res Clin Rheumatol.* Feb 2012;26(1):105-117.

89. Lutman FC, Favata BV. Keratoconjunctivitis sicca and buccoglossopharyngitis sicca with enlargement of parotid glands; report of two cases of Sjogren's syndrome, with pathologic study of a lacrimal gland and the parotid glands in one case. *Arch Ophthal.* Mar 1946;35:227-240.

90. Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjogren's syndrome. *Nat Rev Rheumatol.* Sep 2010;6(9):529-537.

91. Al-Hashimi I. Xerostomia secondary to Sjogren's syndrome in the elderly: recognition and management. *Drugs Aging.* 2005;22(11):887-899.

92. Kittridge A, Routhouska SB, Korman NJ. Dermatologic manifestations of Sjogren syndrome. *J Cutan Med Surg.* Jan-Feb 2011;15(1):8-14.

93. Segal B, Bowman SJ, Fox PC, et al. Primary Sjogren's Syndrome: health experiences and predictors of health quality among patients in the United States. *Health Qual Life Outcomes.* 2009;7:46.

94. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis.* Jun 2002;61(6):554-558.

95. Fox RI, Robinson CA, Curd JG, Kozin F, Howell FV. Sjogren's syndrome. Proposed criteria for classification. *Arthritis Rheum.* May 1986;29(5):577-585.

96. Vitali C, Bombardieri S, Moutsopoulos HM, et al. Preliminary criteria for the classification of Sjogren's syndrome. Results of a prospective concerted action supported by the European Community. *Arthritis Rheum.* Mar 1993;36(3):340-347.

97. Vitali C, Bombardieri S, Moutsopoulos HM, et al. Assessment of the European classification criteria for Sjogren's syndrome in a series of clinically defined cases: results of a prospective multicentre study. The European Study Group on Diagnostic Criteria for Sjogren's Syndrome. *Ann Rheum Dis.* Feb 1996;55(2):116-121.

98. Shiboski SC, Shiboski CH, Criswell L, et al. American College of Rheumatology classification criteria for Sjogren's syndrome: a data-driven, expert consensus approach in the Sjogren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res.* Apr 2012;64(4):475-487.

99. Rasmussen A, Ice JA, Li H, et al. Comparison of the American-European Consensus Group Sjogren's syndrome classification criteria to newly proposed American College of Rheumatology criteria in a large, carefully characterised sicca cohort. *Ann Rheum Dis.* Jan 2014;73(1):31-38.

100. Cornec D, Saraux A, Cochener B, et al. Level of agreement between 2002 American-European Consensus Group and 2012 American College of Rheumatology classification criteria for Sjogren's syndrome and reasons for discrepancies. *Arthritis Res Ther.* 2014;16(2):R74.

101. Hernandez-Molina G, Avila-Casado C, Nunez-Alvarez C, et al. Utility of the American-European Consensus Group and American College of Rheumatology Classification Criteria for Sjogren's syndrome in patients with systemic autoimmune diseases in the clinical setting. *Rheumatology (Oxford).* Mar 2015;54(3):441-448.

102. Sy A, O'Brien KS, Liu MP, et al. Expert opinion in the management of aqueous Deficient Dry Eye Disease (DED). *BMC Ophthalmol.* 2015;15:133.

103. Dartt DA. Neural regulation of lacrimal gland secretory processes: relevance in dry eye diseases. *Prog Retin Eye Res.* May 2009;28(3):155-177.

104. Belmonte C, Aracil A, Acosta MC, Luna C, Gallar J. Nerves and sensations from the eye surface. *Ocul Surf.* Oct 2004;2(4):248-253.

105. Botelho SY. Tears and the Lacrimal Gland. *Sci Am.* Oct 1964;211:78-86.

106. Stern ME, Beuerman RW, Fox RI, Gao J, Mircheff AK, Pflugfelder SC. The pathology of dry eye: the interaction between the ocular surface and lacrimal glands. *Cornea.* Nov 1998;17(6):584-589.

107. Muller LJ, Marfurt CF, Kruse F, Tervo TM. Corneal nerves: structure, contents and function. *Exp Eye Res.* May 2003;76(5):521-542.

108. Snell R. *Clinical Neuroanatomy*. 7th ed. Philadelphia: Wolters Kluwer | Lippincott Williams & Wilkins; 2010.

109. Meneray MA, Bennett DJ, Nguyen DH, Beuerman RW. Effect of sensory denervation on the structure and physiologic responsiveness of rabbit lacrimal gland. *Cornea.* Jan 1998;17(1):99-107.

110. Toshida H, Nguyen DH, Beuerman RW, Murakami A. Evaluation of novel dry eye model: preganglionic parasympathetic denervation in rabbit. *Invest Ophthalmol Vis Sci.* Oct 2007;48(10):4468-4475.

111. Roszkowska AM, Colosi P, Ferreri FM, Galasso S. Age-related modifications of corneal sensitivity. *Ophthalmologica.* Sep-Oct 2004;218(5):350-355.

112. Damato BE, Allan D, Murray SB, Lee WR. Senile atrophy of the human lacrimal gland: the contribution of chronic inflammatory disease. *Br J Ophthalmol.* Sep 1984;68(9):674-680.

113. Rios JD, Horikawa Y, Chen LL, et al. Age-dependent alterations in mouse exorbital lacrimal gland structure, innervation and secretory response. *Exp Eye Res.* Apr 2005;80(4):477-491.

114. Williams RM, Singh J, Sharkey KA. Innervation and mast cells of the rat exorbital lacrimal gland: the effects of age. *J Auton Nerv Syst.* Apr 1994;47(1-2):95-108.

115. Dartt DA. Dysfunctional neural regulation of lacrimal gland secretion and its role in the pathogenesis of dry eye syndromes. *Ocul Surf.* Apr 2004;2(2):76-91.

116. Talsania SD, Robson CD, Mantagos IS. Unilateral Congenital Lacrimal Gland Agenesis With Contralateral Lacrimal Gland Hypoplasia. *J Pediatr Ophthalmol Strabismus.* 2015;52 Online:e52-54.

117. Allgrove J, Clayden GS, Grant DB, Macaulay JC. Familial glucocorticoid deficiency with achalasia of the cardia and deficient tear production. *Lancet.* Jun 17 1978;1(8077):1284-1286.

118. Sarathi V, Shah NS. Triple-A syndrome. *Adv Exp Med Biol.* 2010;685:1-8.

119. Gilbard JP, Rossi SR, Gray KL. A new rabbit model for keratoconjunctivitis sicca. *Invest Ophthalmol Vis Sci.* Feb 1987;28(2):225-228.

120. de Almeida JR, Guyatt GH, Sud S, et al. Management of Bell palsy: clinical practice guideline. *CMAJ.* Sep 2 2014;186(12):917-922.

121. Murthy JM, Saxena AB. Bell's palsy: Treatment guidelines. *Ann Indian Acad Neurol.* Jul 2011;14(Suppl 1):S70-72.

122. Hughes GB. Practical management of Bell's palsy. *Otolaryngol Head Neck Surg.* Jun 1990;102(6):658-663.

123. Oh SH, Lyu B, Yim HB, Lee NY. Lower Lid Laxity is Negatively Correlated with Improvement of the Ocular Surface Disease Index in Dry Eye Treatment. *Curr Eye Res.* Feb 2016;41(2):165-170.

124. Dartt DA, Willcox MD. Complexity of the tear film: importance in homeostasis and dysfunction during disease. *Exp Eye Res.* Dec 2013;117:1-3.

125. Ousler GW, 3rd, Abelson MB, Johnston PR, Rodriguez J, Lane K, Smith LM. Blink patterns and lid-contact times in dry-eye and normal subjects. *Clin Ophthalmol.* 2014;8:869-874.

126. Johnston PR, Rodriguez J, Lane KJ, Ousler G, Abelson MB. The interblink interval in normal and dry eye subjects. *Clin Ophthalmol.* 2013;7:253-259.

127. Holly FJ, Lemp MA. Tear physiology and dry eyes. *Surv Ophthalmol.* Sep-Oct 1977;22(2):69-87.

128. Himebaugh NL, Begley CG, Bradley A, Wilkinson JA. Blinking and tear break-up during four visual tasks. *Optom Vis Sci.* Feb 2009;86(2):E106-114.

129. Chu CA, Rosenfield M, Portello JK. Blink patterns: reading from a computer screen versus hard copy. *Optom Vis Sci.* Mar 2014;91(3):297-302.

130. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1930-1937.

131. Ota Y, Matsumoto Y, Dogru M, et al. Management of evaporative dry eye in ectrodactyly-ectodermal dysplasia-clefting syndrome. *Optom Vis Sci.* Sep 2008;85(9):E795-801.

132. Wali UK, Al-Mujaini A. Sebaceous gland carcinoma of the eyelid. *Oman J Ophthalmol.* Sep 2010;3(3):117-121.

133. Ozdal PC, Codere F, Callejo S, Caissie AL, Burnier MN. Accuracy of the clinical diagnosis of chalazion. *Eye.* Feb 2004;18(2):135-138.

134. Blackie CA, Korb DR, Knop E, Bedi R, Knop N, Holland EJ. Nonobvious obstructive meibomian gland dysfunction. *Cornea.* Dec 2010;29(12):1333-1345.

135. Placzek M, Arnold B, Schmidt H, et al. Elevated 17-hydroxyprogesterone serum values in male patients with acne. *J Am Acad Dermatol.* Dec 2005;53(6):955-958.

136. Zouboulis CC, Chen WC, Thornton MJ, Qin K, Rosenfield R. Sexual hormones in human skin. *Horm Metab Res.* Feb 2007;39(2):85-95.

137. Chen Y, Clegg NJ, Scher HI. Anti-androgens and androgen-depleting therapies in prostate cancer: new agents for an established target. *Lancet Oncol.* Oct 2009;10(10):981-991.

138. Lemay A, Poulin Y. Oral contraceptives as anti-androgenic treatment of acne. *J Obstet Gynaecol Can.* Jul 2002;24(7):559-567.

139. Olami Y, Zajicek G, Cogan M, Gnessin H, Pe'er J. Turnover and migration of meibomian gland cells in rats' eyelids. *Ophthalmic Res.* May-Jun 2001;33(3):170-175.

140. Mauris J, Dieckow J, Schob S, et al. Loss of CD147 results in impaired epithelial cell differentiation and malformation of the meibomian gland. *Cell Death Dis.* 2015;6:e1726.

141. Sullivan DA, Yamagami H, Liu M, et al. Sex steroids, the meibomian gland and evaporative dry eye. *Adv Exp Med Biol.* 2002;506(Pt A):389-399.

142. Simons E, Smith PG. Sensory and autonomic innervation of the rat eyelid: neuronal origins and peptide phenotypes. *J Chem Neuroanat.* Jul 1994;7(1-2):35-47.

143. LeDoux MS, Zhou Q, Murphy RB, Greene ML, Ryan P. Parasympathetic innervation of the meibomian glands in rats. *Invest Ophthalmol Vis Sci.* Oct 2001;42(11):2434-2441.

144. Li L, Jin D, Gao J, et al. Activities of autonomic neurotransmitters in Meibomian gland tissues are associated with menopausal dry eye. *Neural Regen Res.* Dec 15 2012;7(35):2761-2769.

145. Jester JV, Nicolaides N, Smith RE. Meibomian gland dysfunction. I. Keratin protein expression in normal human and rabbit meibomian glands. *Invest Ophthalmol Vis Sci.* May 1989;30(5):927-935.

146. Krenzer KL, Dana MR, Ullman MD, et al. Effect of androgen deficiency on the human meibomian gland and ocular surface. *J Clin Endocrinol Metab.* Dec 2000;85(12):4874-4882.

147. Korb DR, Henriquez AS. Meibomian gland dysfunction and contact lens intolerance. *J Am Optom Assoc.* Mar 1980;51(3):243-251.

148. Jester JV, Nicolaides N, Kiss-Palvolgyi I, Smith RE. Meibomian gland dysfunction. II. The role of keratinization in a rabbit model of MGD. *Invest Ophthalmol Vis Sci.* May 1989;30(5):936-945.

149. Hykin PG, Bron AJ. Age-related morphological changes in lid margin and meibomian gland anatomy. *Cornea.* Jul 1992;11(4):334-342.

150. Nichols KK, Hanlon SD, Nichols JJ. A Murine Model for Characterizing Glandular Changes in Obstructive Meibomian Gland Dysfunction. *Invest Ophthalmol Vis Sci.* 2014;55(13):14-14.

151. Jester JV, Rife L, Nii D, Luttrull JK, Wilson L, Smith RE. In vivo biomicroscopy and photography of meibomian glands in a rabbit model of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci.* May 1982;22(5):660-667.

152. Parfitt GJ, Xie Y, Geyfman M, Brown DJ, Jester JV. Absence of ductal hyper-keratinization in mouse age-related meibomian gland dysfunction (ARMGD). *Aging.* Nov 2013;5(11):825-834.

153. Pucker AD, Nichols JJ. Analysis of meibum and tear lipids. *Ocul Surf.* Oct 2012;10(4):230-250.

154. Chew CK, Hykin PG, Jansweijer C, Dikstein S, Tiffany JM, Bron AJ. The casual level of meibomian lipids in humans. *Curr Eye Res.* Mar 1993;12(3):255-259.

155. Borchman D, Foulks GN, Yappert MC, et al. Physical changes in human meibum with age as measured by infrared spectroscopy. *Ophthalmic Res.* 2010;44(1):34-42.

156. Borchman D, Foulks GN, Yappert MC, Milliner SE. Changes in human meibum lipid composition with age using nuclear magnetic resonance spectroscopy. *Invest Ophthalmol Vis Sci.* Jan 2012;53(1):475-482.

157. Borchman D, Foulks GN, Yappert MC, et al. Human meibum lipid conformation and thermodynamic changes with meibomian-gland dysfunction. *Invest Ophthalmol Vis Sci.* May 2011;52(6):3805-3817.

158. Geerling G, Tauber J, Baudouin C, et al. The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):2050-2064.

159. Butovich IA, Lu H, McMahon A, et al. Biophysical and morphological evaluation of human normal and dry eye meibum using hot stage polarized light microscopy. *Invest Ophthalmol Vis Sci.* Jan 2014;55(1):87-101.

160. King-Smith PE, Bailey MD, Braun RJ. Four characteristics and a model of an effective tear film lipid layer (TFLL). *Ocul Surf.* Oct 2013;11(4):236-245.

161. Millar TJ, Schuett BS. The real reason for having a meibomian lipid layer covering the outer surface of the tear film - A review. *Exp Eye Res.* Aug 2015;137:125-138.

162. Schaumberg DA, 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.* Mar 2011;52(4):1994-2005.

163. Lemp MA, Crews LA, Bron AJ, Foulks GN, Sullivan BD. Distribution of aqueous-deficient and evaporative dry eye in a clinic-based patient cohort: a retrospective study. *Cornea.* May 2012;31(5):472-478.

164. Hingorani M, Hanson I, van Heyningen V. Aniridia. *Eur J Hum Genet.* Oct 2012;20(10):1011-1017.

165. Call M, Fischesser K, Lunn MO, Kao WW. A unique lineage gives rise to the meibomian gland. *Mol Vis.* 2016;22:168-176.

166. Megarbane H, Megarbane A. Ichthyosis follicularis, alopecia, and photophobia (IFAP) syndrome. *Orphanet J Rare Dis.* 2011;6:29.

167. Fatima T, Mathur U, Acharya M. Meibomian gland dysfunction in a case of ichthyosis follicularis with alopecia and photophobia syndrome. *Indian J Ophthalmol.* Mar 2014;62(3):365-367.

168. Kojima T, Dogru M, Matsumoto Y, Goto E, Tsubota K. Tear film and ocular surface abnormalities after eyelid tattooing. *Ophthal Plast Reconstr Surg.* Jan 2005;21(1):69-71.

169. Lee YB, Kim JJ, Hyon JY, Wee WR, Shin YJ. Eyelid Tattooing Induces Meibomian Gland Loss and Tear Film Instability. *Cornea.* Jul 2015;34(7):750-755.

170. Gonnering RS, Sonneland PR. Meibomian gland dysfunction in floppy eyelid syndrome. *Ophthal Plast Reconstr Surg.* 1987;3(2):99-103.

171. Netland PA, Sugrue SP, Albert DM, Shore JW. Histopathologic features of the floppy eyelid syndrome. Involvement of tarsal elastin. *Ophthalmology.* Jan 1994;101(1):174-181.

172. Ong BL. Relation between contact lens wear and Meibomian gland dysfunction. *Optom Vis Sci.* Mar 1996;73(3):208-210.

173. Arita R, Itoh K, Inoue K, Kuchiba A, Yamaguchi T, Amano S. Contact lens wear is associated with decrease of meibomian glands. *Ophthalmology.* Mar 2009;116(3):379-384.

174. Marren SE. Contact lens wear, use of eye cosmetics, and Meibomian gland dysfunction. *Optom Vis Sci.* Jan 1994;71(1):60-62.

175. Arita R, Itoh K, Maeda S, Maeda K, Tomidokoro A, Amano S. Association of contact lens-related allergic conjunctivitis with changes in the morphology of meibomian glands. *Jpn J Ophthalmol.* Jan 2012;56(1):14-19.

176. Machalinska A, Zakrzewska A, Adamek B, et al. Comparison of Morphological and Functional Meibomian Gland Characteristics Between Daily Contact Lens Wearers and Nonwearers. *Cornea.* Sep 2015;34(9):1098-1104.

177. Pucker AD, Jones-Jordan LA, Li W, et al. Associations with Meibomian Gland Atrophy in Daily Contact Lens Wearers. *Optom Vis Sci.* Sep 2015;92(9):e206-213.

178. Pult H, Purslow C, Murphy PJ. The relationship between clinical signs and dry eye symptoms. *Eye.* Apr 2011;25(4):502-510.

179. Sullivan BD, Crews LA, Messmer EM, et al. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications. *Acta Ophthalmol.* Mar 2014;92(2):161-166.

180. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. *Cornea.* Nov 2004;23(8):762-770.

181. Ngo W, Situ P, Keir N, Korb D, Blackie C, Simpson T. Psychometric properties and validation of the Standard Patient Evaluation of Eye Dryness questionnaire. *Cornea.* Sep 2013;32(9):1204-1210.

182. Schaumberg DA, Gulati A, Mathers WD, et al. Development and validation of a short global dry eye symptom index. *Ocul Surf.* Jan 2007;5(1):50-57.

183. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* May 2000;118(5):615-621.

184. Abetz L, Rajagopalan K, Mertzanis P, Begley C, Barnes R, Chalmers R. Development and validation of the impact of dry eye on everyday life (IDEEL) questionnaire, a patient-reported outcomes (PRO) measure for the assessment of the burden of dry eye on patients. *Health Qual Life Outcomes.* 2011;9:111.

185. Streiner D, Norman G. *Health Measurement Scales: A Practical Guide to Their Development and Use*. 3rd ed. New York, NY: Oxford University Press; 2003.

186. Bond T, Fox C. *Applying the Rasch Model: Fundamental Measurement in the Human Sciences*. 2nd ed. New York, NY: Routledge; 2007.

187. Dougherty BE, Nichols JJ, Nichols KK. Rasch analysis of the Ocular Surface Disease Index (OSDI). *Invest Ophthalmol Vis Sci.* Nov 2011;52(12):8630-8635.

188. Grubbs JR, Jr., Tolleson-Rinehart S, Huynh K, Davis RM. A review of quality of life measures in dry eye questionnaires. *Cornea.* Feb 2014;33(2):215-218.

189. Johnson ME, Murphy PJ. Measurement of ocular surface irritation on a linear interval scale with the ocular comfort index. *Invest Ophthalmol Vis Sci.* Oct 2007;48(10):4451-4458.

190. Ornek N, Karabulut AA, Ornek K, Onaran Z, Usta G. Corneal and conjunctival sensitivity in rosacea patients. *Saudi J Ophthalmol.* Jan-Mar 2016;30(1):29-32.

191. DeMill DL, Hussain M, Pop-Busui R, Shtein RM. Ocular surface disease in patients with diabetic peripheral neuropathy. *Br J Ophthalmol.* Oct 23 2015.

192. Hallak JA, Tibrewal S, Jain S. Depressive Symptoms in Patients With Dry Eye Disease: A Case-Control Study Using the Beck Depression Inventory. *Cornea.* Dec 2015;34(12):1545-1550.

193. Amparo F, Schaumberg DA, Dana R. Comparison of Two Questionnaires for Dry Eye Symptom Assessment: The Ocular Surface Disease Index and the Symptom Assessment in Dry Eye. *Ophthalmology.* Jul 2015;122(7):1498-1503.

194. Bron AJ, Abelson MB, Ousler G, et al. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):108-152.

195. Igarashi T, Fujimoto C, Suzuki H, Ono M, Iijima O, Takahashi H. Short-time exposure of hyperosmolarity triggers interleukin-6 expression in corneal epithelial cells. *Cornea.* Dec 2014;33(12):1342-1347.

196. Bunya VY, Langelier N, Chen S, Pistilli M, Vivino FB, Massaro-Giordano G. Tear osmolarity in Sjogren syndrome. *Cornea.* Jul 2013;32(7):922-927.

197. Pena-Verdeal H, Garcia-Resua C, Minones M, Giraldez MJ, Yebra-Pimentel E. Accuracy of a Freezing Point Depression Technique Osmometer. *Optom Vis Sci.* Sep 2015;92(9):e273-283.

198. Gokhale M, Stahl U, Jalbert I. In situ osmometry: validation and effect of sample collection technique. *Optom Vis Sci.* Apr 2013;90(4):359-365.

199. Tomlinson A, McCann LC, Pearce EI. Comparison of human tear film osmolarity measured by electrical impedance and freezing point depression techniques. *Cornea.* Sep 2010;29(9):1036-1041.

200. Terry JE, Hill RM. Human tear osmotic pressure: diurnal variations and the closed eye. *Arch Ophthalmol.* Jan 1978;96(1):120-122.

201. Pensyl CD, Benjamin WJ. Vapor pressure osmometry: minimum sample microvolumes. *Acta Ophthalmol Scand.* Feb 1999;77(1):27-30.

202. Versura P, Campos EC. TearLab(R) Osmolarity System for diagnosing dry eye. *Expert Rev Mol Diagn.* Mar 2013;13(2):119-129.

203. Khanal S, Tomlinson A, McFadyen A, Diaper C, Ramaesh K. Dry eye diagnosis. *Invest Ophthalmol Vis Sci.* Apr 2008;49(4):1407-1414.

204. Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: determination of a referent for dry eye diagnosis. *Invest Ophthalmol Vis Sci.* Oct 2006;47(10):4309-4315.

205. Bunya VY, Fuerst NM, Pistilli M, et al. Variability of Tear Osmolarity in Patients With Dry Eye. *JAMA Ophthalmol.* Jun 2015;133(6):662-667.

206. Garcia-Resua C, Pena-Verdeal H, Remeseiro B, Giraldez MJ, Yebra-Pimentel E. Correlation between tear osmolarity and tear meniscus. *Optom Vis Sci.* Dec 2014;91(12):1419-1429.

207. Hassan Z, Szalai E, Berta A, Modis L, Jr., Nemeth G. Assessment of tear osmolarity and other dry eye parameters in post-LASIK eyes. *Cornea.* Jul 2013;32(7):e142-145.

208. Caffery B, Chalmers RL, Marsden H, et al. Correlation of tear osmolarity and dry eye symptoms in convention attendees. *Optom Vis Sci.* Feb 2014;91(2):142-149.

209. Chen SP, Massaro-Giordano G, Pistilli M, Schreiber CA, Bunya VY. Tear osmolarity and dry eye symptoms in women using oral contraception and contact lenses. *Cornea.* Apr 2013;32(4):423-428.

210. Sweeney DF, Millar TJ, Raju SR. Tear film stability: a review. *Exp Eye Res.* Dec 2013;117:28-38.

211. Johnson ME, Murphy PJ. The Effect of instilled fluorescein solution volume on the values and repeatability of TBUT measurements. *Cornea.* Oct 2005;24(7):811-817.

212. Korb DR, Greiner JV, Herman J. Comparison of fluorescein break-up time measurement reproducibility using standard fluorescein strips versus the Dry Eye Test (DET) method. *Cornea.* Nov 2001;20(8):811-815.

213. Marquardt R, Stodtmeiser R, Christ T. Modification of tear film break-up time test for increased reliability. *The Preocular Tear Film in Health, Disease and Contact Lens Wear. Lubbock, Texas: Dry Eye Institute.* 1986:57-63.

214. Mengher LS, Bron AJ, Tonge SR, Gilbert DJ. Effect of fluorescein instillation on the pre-corneal tear film stability. *Curr Eye Res.* Jan 1985;4(1):9-12.

215. Cho P, Douthwaite W. The relation between invasive and noninvasive tear break-up time. *Optom Vis Sci.* Jan 1995;72(1):17-22.

216. Gumus K, Crockett CH, Rao K, et al. Noninvasive assessment of tear stability with the tear stability analysis system in tear dysfunction patients. *Invest Ophthalmol Vis Sci.* Jan 2011;52(1):456-461.

217. Gaw DB, Tinio BGO. A Simplified Xeroscope for the Noninvasive Measurement of Tear Break-up Time. *Philipp J Ophthalmol.* 2015;40:18-23.

218. Butovich IA. The Meibomian puzzle: combining pieces together. *Prog Retin Eye Res.* Nov 2009;28(6):483-498.

219. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. *Optom Vis Sci.* Jan 1997;74(1):8-13.

220. Korb DR, Baron DF, Herman JP, et al. Tear film lipid layer thickness as a function of blinking. *Cornea.* Jul 1994;13(4):354-359.

221. Isreb MA, Greiner JV, Korb DR, et al. Correlation of lipid layer thickness measurements with fluorescein tear film break-up time and Schirmer's test. *Eye.* Jan 2003;17(1):79-83.

222. Blackie CA, Solomon JD, Scaffidi RC, Greiner JV, Lemp MA, Korb DR. The relationship between dry eye symptoms and lipid layer thickness. *Cornea.* Aug 2009;28(7):789-794.

223. Finis D, Pischel N, Schrader S, Geerling G. Evaluation of lipid layer thickness measurement of the tear film as a diagnostic tool for Meibomian gland dysfunction. *Cornea.* Dec 2013;32(12):1549-1553.

224. Satjawatcharaphong P, Ge S, Lin MC. Clinical Outcomes Associated with Thermal Pulsation System Treatment. *Optom Vis Sci.* Sep 2015;92(9):e334-341.

225. Zhao Y, Tan CL, Tong L. Intra-observer and inter-observer repeatability of ocular surface interferometer in measuring lipid layer thickness. *BMC Ophthalmol.* 2015;15:53.

226. Blackie CA, Korb DR. Recovery time of an optimally secreting meibomian gland. *Cornea.* Apr 2009;28(3):293-297.

227. Friedland BR, Fleming CP, Blackie CA, Korb DR. A novel thermodynamic treatment for meibomian gland dysfunction. *Curr Eye Res.* Feb 2011;36(2):79-87.

228. Greiner JV. A single LipiFlow(R) Thermal Pulsation System treatment improves meibomian gland function and reduces dry eye symptoms for 9 months. *Curr Eye Res.* Apr 2012;37(4):272-278.

229. Paugh J, Kwan JT, Nguyen A, Senchyna M, Christensen M, Meadows D. Comparison of Upper and Lower Eyelid Meibomian Glands: Are They Different? *Optom Vis Sci.* 2011;88:E-abstract 115721.

230. Mathers WD, Shields WJ, Sachdev MS, Petroll WM, Jester JV. Meibomian gland dysfunction in chronic blepharitis. *Cornea.* Jul 1991;10(4):277-285.

231. Bron AJ, Benjamin L, Snibson GR. Meibomian gland disease. Classification and grading of lid changes. *Eye.* 1991;5 (Pt 4):395-411.

232. Shimazaki J, Sakata M, Tsubota K. Ocular surface changes and discomfort in patients with meibomian gland dysfunction. *Arch Ophthalmol.* Oct 1995;113(10):1266-1270.

233. Ngo W, Srinivasan S, Jones L. Historical overview of imaging the meibomian glands. *Journal of optometry.* 2013;6(1):1-8.

234. Tapie R. Etude biomicroscopique des glandes de meibomius. *Annales d'Oculistique.* 1977;210(9):637-648.

235. Robin JB, Jester JV, Nobe J, Nicolaides N, Smith RE. In vivo transillumination biomicroscopy and photography of meibomian gland dysfunction. A clinical study. *Ophthalmology.* Oct 1985;92(10):1423-1426.

236. Jester JV, Rife L, Nii D, Luttrull JK, Wilson L, Smith RE. In vivo biomicroscopy and photography of meibomian glands in a rabbit model of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci.* 1982;22(5):660-667.

237. Yokoi N, Komuro A, Yamada H, Maruyama K, Kinoshita S. A newly developed video-meibography system featuring a newly designed probe. *Japanese journal of ophthalmology.* 2007;51(1):53-56.

238. Shimazaki J, Goto E, Ono M, Shimmura S, Tsubota K. Meibomian gland dysfunction in patients with Sjogren syndrome. *Ophthalmology.* Aug 1998;105(8):1485-1488.

239. McCann LC, Tomlinson A, Pearce EI, Diaper C. Tear and meibomian gland function in blepharitis and normals. *Eye Contact Lens.* Jul 2009;35(4):203-208.

240. Goto E, Endo K, Suzuki A, Fujikura Y, Tsubota K. Improvement of tear stability following warm compression in patients with meibomian gland dysfunction. *Adv Exp Med Biol.* 2002;506(Pt B):1149-1152.

241. Nichols JJ, Berntsen DA, Mitchell GL, Nichols KK. An assessment of grading scales for meibography images. *Cornea.* May 2005;24(4):382-388.

242. Den S, Shimizu K, Ikeda T, Tsubota K, Shimmura S, Shimazaki J. Association between meibomian gland changes and aging, sex, or tear function. *Cornea.* Jul 2006;25(6):651-655.

243. Mathers WD, Daley T, Verdick R. Video imaging of the meibomian gland. *Arch Ophthalmol.* Apr 1994;112(4):448-449.

244. Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology.* May 2008;115(5):911-915.

245. Pult H, Riede-Pult BH. Non-contact meibography: keep it simple but effective. *Cont Lens Anterior Eye.* Apr 2012;35(2):77-80.

246. Srinivasan S, Menzies K, Sorbara L, Jones L. Meibography of the Upper Lid. *Optician.* 2011:12-14. https://www.opticianonline.net/features/meibography-of-the-upper-lid. Accessed January 2012.

247. Srinivasan S, Sorbara L, Jones L, Sickenberger W. Imaging the Structure of the Meibomian Glands. *Contact Lens Spectrum.* 2011(July):52-53. http://www.clspectrum.com/articleviewer.aspx?articleID=105800. Accessed January 2012.

248. Ngo W, Srinivasan S, Schulze M, Jones L. Repeatability of grading meibomian gland dropout using two infrared systems. *Optom Vis Sci.* Jun 2014;91(6):658-667.

249. Arita R, Itoh K, Maeda S, et al. Meibomian gland duct distortion in patients with perennial allergic conjunctivitis. *Cornea.* Aug 2010;29(8):858-860.

250. Pult H, Riede-Pult BH, Nichols JJ. Relation between upper and lower lids' meibomian gland morphology, tear film, and dry eye. *Optom Vis Sci.* Mar 2012;89(3):E310-315.

251. Arita R, Itoh K, Maeda S, et al. Proposed diagnostic criteria for obstructive meibomian gland dysfunction. *Ophthalmology.* Nov 2009;116(11):2058-2063 e2051.

252. Arita R, Itoh K, Maeda S, et al. Proposed diagnostic criteria for seborrheic meibomian gland dysfunction. *Cornea.* Sep 2010;29(9):980-984.

253. Arita R, Itoh K, Maeda S, Maeda K, Tomidokoro A, Amano S. Efficacy of diagnostic criteria for the differential diagnosis between obstructive meibomian gland dysfunction and aqueous deficiency dry eye. *Jpn J Ophthalmol.* Sep 2010;54(5):387-391.

254. TearScience. Evaluate | TearScience. 2016; http://tearscience.com/en/the-tearscience-system/evaluate/. Accessed 03 June 2016.

255. Jester JV, Nicolaides N, Smith RE. Meibomian gland studies: histologic and ultrastructural investigations. *Invest Ophthalmol Vis Sci.* Apr 1981;20(4):537-547.

256. Sirigu P, Shen RL, Pinto da Silva P. Human meibomian glands: the ultrastructure of acinar cells as viewed by thin section and freeze-fracture transmission electron microscopies. *Invest Ophthalmol Vis Sci.* Jun 1992;33(7):2284-2292.

257. Heidelberg. Rostock Cornea Module - Quick Operation Notes. 2012:1-2. http://www.heidelbergengineering.com/us/wp-content/uploads/97097-rcmquickusernotes_031605.pdf. Accessed January 2012.

258. Heidelberg. Confocal Laser Microscopy. 2005:1-6. http://www.heidelbergengineering.com/wp-content/uploads/665-hrt-rostock-cornea-module.pdf. Accessed January 2012.

259. Bizheva K, Lee P, Sorbara L, Hutchings N, Simpson T. In vivo volumetric imaging of the human upper eyelid with ultrahigh-resolution optical coherence tomography. *J Biomed Opt.* Jul-Aug 2010;15(4):040508.

260. Kobayashi A, Yoshita T, Sugiyama K. In vivo findings of the bulbar/palpebral conjunctiva and presumed meibomian glands by laser scanning confocal microscopy. *Cornea.* Nov 2005;24(8):985-988.

261. Efron N, Al-Dossari M, Pritchard N. In vivo confocal microscopy of the palpebral conjunctiva and tarsal plate. *Optom Vis Sci.* Nov 2009;86(11):E1303-1308.

262. Sorbara L, Maram J, Bizheva K, Hutchings N, Simpson TL. Case report: Chalazion and its features visualized by ultrahigh resolution optical coherence tomography. *Cont Lens Anterior Eye.* Apr 2011;34(2):87-91.

263. Peyman GA, Ingram CP, Montilla LG, Witte RS. A high-resolution 3D ultrasonic system for rapid evaluation of the anterior and posterior segment. *Ophthalmic Surg Lasers Imaging.* Mar-Apr 2012;43(2):143-151.

264. Ibrahim OM, Matsumoto Y, Dogru M, et al. The efficacy, sensitivity, and specificity of in vivo laser confocal microscopy in the diagnosis of meibomian gland dysfunction. *Ophthalmology.* Apr 2010;117(4):665-672.

265. Matsumoto Y, Sato EA, Ibrahim OM, Dogru M, Tsubota K. The application of in vivo laser confocal microscopy to the diagnosis and evaluation of meibomian gland dysfunction. *Mol Vis.* 2008;14:1263-1271.

266. Matsumoto Y, Shigeno Y, Sato EA, et al. The evaluation of the treatment response in obstructive meibomian gland disease by in vivo laser confocal microscopy. *Graefes Arch Clin Exp Ophthalmol.* Jun 2009;247(6):821-829.

267. Wei A, Hong J, Sun X, Xu J. Evaluation of age-related changes in human palpebral conjunctiva and meibomian glands by in vivo confocal microscopy. *Cornea.* Sep 2011;30(9):1007-1012.

268. Villani E, Ceresara G, Beretta S, Magnani F, Viola F, Ratiglia R. In vivo confocal microscopy of meibomian glands in contact lens wearers. *Invest Ophthalmol Vis Sci.* Jul 2011;52(8):5215-5219.

269. Villani E, Beretta S, De Capitani M, Galimberti D, Viola F, Ratiglia R. In vivo confocal microscopy of meibomian glands in Sjogren's syndrome. *Invest Ophthalmol Vis Sci.* Feb 2011;52(2):933-939.

270. Hwang HS, Shin JG, Lee BH, Eom TJ, Joo CK. In Vivo 3D Meibography of the Human Eyelid Using Real Time Imaging Fourier-Domain OCT. *PLoS One.* 2013;8(6):e67143.

271. Hwang HS, Park CW, Joo CK. Novel noncontact meibography with anterior segment optical coherence tomography: Hosik meibography. *Cornea.* Jan 2013;32(1):40-43.

272. Drexler W, G. FJ. *Optical Coherence Tomography: Technology and Applications*. Berlin: Springer-Verlag; 2008.

273. Ju MJ, Shin JG, Hoshi S, et al. Three-dimensional volumetric human meibomian gland investigation using polarization-sensitive optical coherence tomography. *J Biomed Opt.* Mar 2014;19(3):30503.

274. McCulley JP, Shine WE, Aronowicz J, Oral D, Vargas J. Presumed hyposecretory/hyperevaporative KCS: tear characteristics. *Transactions of the American Ophthalmological Society.* 2003;101:141-152; discussion 152-144.

275. Aronowicz JD, Shine WE, Oral D, Vargas JM, McCulley JP. Short term oral minocycline treatment of meibomianitis. *Br J Ophthalmol.* Jul 2006;90(7):856-860.

276. Goto E, Monden Y, Takano Y, et al. Treatment of non-inflamed obstructive meibomian gland dysfunction by an infrared warm compression device. *Br J Ophthalmol.* Dec 2002;86(12):1403-1407.

277. Srinivasan S, Menzies K, Sorbara L, Jones L. Infra-red imaging of meibomian gland structure using a novel keratograph. *Optom Vis Sci.* May 2012;89(5):788-794.

278. Bailey IL, Bullimore MA, Raasch TW, Taylor HR. Clinical grading and the effects of scaling. *Invest Ophthalmol Vis Sci.* Feb 1991;32(2):422-432.

279. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):2006-2049.

280. Morgan PB, Maldonado-Codina C. Corneal staining: do we really understand what we are seeing? *Cont Lens Anterior Eye.* Apr 2009;32(2):48-54.

281. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea.* Oct 2003;22(7):640-650.

282. Lemp MA. Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes. *CLAO J.* Oct 1995;21(4):221-232.

283. Miyata K, Amano S, Sawa M, Nishida T. A novel grading method for superficial punctate keratopathy magnitude and its correlation with corneal epithelial permeability. *Arch Ophthalmol.* Nov 2003;121(11):1537-1539.

284. Whitcher JP, Shiboski CH, Shiboski SC, et al. A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjogren's Syndrome International Registry. *Am J Ophthalmol.* Mar 2010;149(3):405-415.

285. Sook Chun Y, Park IK. Reliability of 4 clinical grading systems for corneal staining. *Am J Ophthalmol.* May 2014;157(5):1097-1102.

286. Chun YS, Yoon WB, Kim KG, Park IK. Objective assessment of corneal staining using digital image analysis. *Invest Ophthalmol Vis Sci.* Dec 2014;55(12):7896-7903.

287. Rodriguez JD, Lane KJ, Ousler GW, 3rd, Angjeli E, Smith LM, Abelson MB. Automated Grading System for Evaluation of Superficial Punctate Keratitis Associated With Dry Eye. *Invest Ophthalmol Vis Sci.* Apr 2015;56(4):2340-2347.

288. Zeev MS, Miller DD, Latkany R. Diagnosis of dry eye disease and emerging technologies. *Clin Ophthalmol.* 2014;8:581-590.

289. Bitton E, Wittich W. Influence of eye position on the Schirmer tear test. *Cont Lens Anterior Eye.* Aug 2014;37(4):257-261.

290. Serin D, Karsloglu S, Kyan A, Alagoz G. A simple approach to the repeatability of the Schirmer test without anesthesia: eyes open or closed? *Cornea.* Sep 2007;26(8):903-906.

291. Bunya VY, Brainard DH, Daniel E, et al. Assessment of signs of anterior blepharitis using standardized color photographs. *Cornea.* Nov 2013;32(11):1475-1482.

292. 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.

293. Bron AJ, Yokoi N, Gaffney EA, Tiffany JM. A solute gradient in the tear meniscus. I. A hypothesis to explain Marx's line. *Ocul Surf.* Apr 2011;9(2):70-91.

294. Kim S, Blackie CA, Korb DR. Age related anteroplacement of the line of marx and contact lens wear. *Optom Vis Sci.* 2012;89:E-abstract 125380.

295. Yamaguchi M, Kutsuna M, Uno T, Zheng X, Kodama T, Ohashi Y. Marx line: fluorescein staining line on the inner lid as indicator of meibomian gland function. *Am J Ophthalmol.* Apr 2006;141(4):669-675.

296. Doughty MJ, Naase T, Donald C, Hamilton L, Button NF. Visualisation of "Marx's line" along the marginal eyelid conjunctiva of human subjects with lissamine green dye. *Ophthalmic Physiol Opt.* Jan 2004;24(1):1-7.

297. Korb DR, Blackie CA. Debridement-scaling: a new procedure that increases Meibomian gland function and reduces dry eye symptoms. *Cornea.* Dec 2013;32(12):1554-1557.

298. Ngo W, Caffery B, Srinivasan S, Jones LW. Effect of Lid Debridement-Scaling in Sjogren Syndrome Dry Eye. *Optom Vis Sci.* Sep 2015;92(9):e316-320.

299. Knop E, Knop N, Zhivov A, et al. The lid wiper and muco-cutaneous junction anatomy of the human eyelid margins: an in vivo confocal and histological study. *J Anat.* Apr 2011;218(4):449-461.

300. Korb DR, Herman JP, Greiner JV, et al. Lid wiper epitheliopathy and dry eye symptoms. *Eye Contact Lens.* Jan 2005;31(1):2-8.

301. Pflugfelder SC, Geerling G, Kinoshita S, et al. Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):163-178.

302. Vibhute S, Kawtikwar P, Kshirsagar S, Sakarkar D. Formulation and evaluation of tear substitutes. *Int J Pharm Sci Rev Res.* 2010;2:17-20.

303. Aguilar AJ, Marquez MI, Albera PA, Tredicce JL, Berra A. Effects of Systane((R)) Balance on noninvasive tear film break-up time in patients with lipid-deficient dry eye. *Clin Ophthalmol.* 2014;8:2365-2372.

304. Simmons PA, Carlisle-Wilcox C, Vehige JG. Comparison of novel lipid-based eye drops with aqueous eye drops for dry eye: a multicenter, randomized controlled trial. *Clin Ophthalmol.* 2015;9:657-664.

305. Simmons PA, Liu H, Carlisle-Wilcox C, Vehige JG. Efficacy and safety of two new formulations of artificial tears in subjects with dry eye disease: a 3-month, multicenter, active-controlled, randomized trial. *Clin Ophthalmol.* 2015;9:665-675.

306. Pinto-Fraga J, Lopez-de la Rosa A, Blazquez Arauzo F, Urbano Rodriguez R, Gonzalez-Garcia MJ. Efficacy and Safety of 0.2% Hyaluronic Acid in the Management of Dry Eye Disease. *Eye Contact Lens.* Jan 16 2016.

307. Berger JS, Head KR, Salmon TO. Comparison of two artificial tear formulations using aberrometry. *Clin Exp Optom.* May 2009;92(3):206-211.

308. Prabhasawat P, Ruangvaravate N, Tesavibul N, Thewthong M. Effect of 0.3% Hydroxypropyl Methylcellulose/Dextran Versus 0.18% Sodium Hyaluronate in the Treatment of Ocular Surface Disease in Glaucoma Patients: A Randomized, Double-Blind, and Controlled Study. *J Ocul Pharmacol Ther.* Jul-Aug 2015;31(6):323-329.

309. Murakami DK, Blackie CA, Korb DR. All Warm Compresses Are Not Equally Efficacious. *Optom Vis Sci.* Sep 2015;92(9):e327-333.

310. Sim HS, Petznick A, Barbier S, et al. A Randomized, Controlled Treatment Trial of Eyelid-Warming Therapies in Meibomian Gland Dysfunction. *Ophthalmol Ther.* Dec 2014;3(1-2):37-48.

311. Bilkhu PS, Naroo SA, Wolffsohn JS. Randomised masked clinical trial of the MGDRx EyeBag for the treatment of meibomian gland dysfunction-related evaporative dry eye. *Br J Ophthalmol.* Dec 2014;98(12):1707-1711.

312. Blackie CA, Carlson AN, Korb DR. Treatment for meibomian gland dysfunction and dry eye symptoms with a single-dose vectored thermal pulsation: a review. *Curr Opin Ophthalmol.* Jul 2015;26(4):306-313.

313. Nagymihalyi A, Dikstein S, Tiffany JM. The influence of eyelid temperature on the delivery of meibomian oil. *Exp Eye Res.* Mar 2004;78(3):367-370.

314. Blackie CA, Solomon JD, Greiner JV, Holmes M, Korb DR. Inner eyelid surface temperature as a function of warm compress methodology. *Optom Vis Sci.* Aug 2008;85(8):675-683.

315. Lacroix Z, Leger S, Bitton E. Ex vivo heat retention of different eyelid warming masks. *Cont Lens Anterior Eye.* Jun 2015;38(3):152-156.

316. Benitez Del Castillo JM, Kaercher T, Mansour K, Wylegala E, Dua H. Evaluation of the efficacy, safety, and acceptability of an eyelid warming device for the treatment of meibomian gland dysfunction. *Clin Ophthalmol.* 2014;8:2019-2027.

317. Doan S, Chiambaretta F, Baudouin C. Evaluation of an eyelid warming device (Blephasteam) for the management of ocular surface diseases in France: the ESPOIR study. *J Fr Ophtalmol.* Dec 2014;37(10):763-772.

318. Villani E, Garoli E, Canton V, Pichi F, Nucci P, Ratiglia R. Evaluation of a novel eyelid-warming device in meibomian gland dysfunction unresponsive to traditional warm compress treatment: an in vivo confocal study. *Int Ophthalmol.* Jun 2015;35(3):319-323.

319. Mori A, Shimazaki J, Shimmura S, Fujishima H, Oguchi Y, Tsubota K. Disposable eyelid-warming device for the treatment of meibomian gland dysfunction. *Jpn J Ophthalmol.* Nov-Dec 2003;47(6):578-586.

320. Paliwoda RE, Newbigging AM, Wang Z, Le XC. Benefits and risks associated with consumption of Great Lakes fish containing omega-3 fatty acids and polychlorinated biphenyls (PCBs). *J Environ Sci (China).* Mar 2016;41:1-5.

321. Rosenblat JD, Kakar R, Berk M, et al. Anti-inflammatory agents in the treatment of bipolar depression: a systematic review and meta-analysis. *Bipolar Disord.* Mar 2016;18(2):89-101.

322. Tajuddin N, Shaikh A, Hassan A. Prescription omega-3 fatty acid products: considerations for patients with diabetes mellitus. *Diabetes Metab Syndr Obes.* 2016;9:109-118.

323. Sperling LS, Nelson JR. History and future of omega-3 fatty acids in cardiovascular disease. *Curr Med Res Opin.* 2016;32(2):301-311.

324. Rizos EC, Elisaf MS. Current evidence and future perspectives of omega-3 polyunsaturated fatty acids for the prevention of cardiovascular disease. *Eur J Pharmacol.* Apr 15 2013;706(1-3):1-3.

325. Hill CL, March LM, Aitken D, et al. Fish oil in knee osteoarthritis: a randomised clinical trial of low dose versus high dose. *Ann Rheum Dis.* Jan 2016;75(1):23-29.

326. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr.* Dec 2002;21(6):495-505.

327. Surette ME. The science behind dietary omega-3 fatty acids. *CMAJ.* Jan 15 2008;178(2):177-180.

328. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr.* Jun 2006;83(6 Suppl):1505S-1519S.

329. Umemoto N, Ishii H, Kamoi D, et al. Reverse association of omega-3/omega-6 polyunsaturated fatty acids ratios with carotid atherosclerosis in patients on hemodialysis. *Atherosclerosis.* Apr 1 2016;249:65-69.

330. Suda S, Katsumata T, Okubo S, et al. Low serum n-3 polyunsaturated fatty acid/n-6 polyunsaturated fatty acid ratio predicts neurological deterioration in Japanese patients with acute ischemic stroke. *Cerebrovasc Dis.* 2013;36(5-6):388-393.

331. Nishizaki Y, Shimada K, Tani S, et al. Significance of imbalance in the ratio of serum n-3 to n-6 polyunsaturated fatty acids in patients with acute coronary syndrome. *Am J Cardiol.* Feb 1 2014;113(3):441-445.

332. Miljanovic B, Trivedi KA, Dana MR, Gilbard JP, Buring JE, Schaumberg DA. Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. *Am J Clin Nutr.* Oct 2005;82(4):887-893.

333. Amparo F, Dastjerdi MH, Okanobo A, et al. Topical interleukin 1 receptor antagonist for treatment of dry eye disease: a randomized clinical trial. *JAMA Ophthalmol.* Jun 2013;131(6):715-723.

334. Galbis-Estrada C, Pinazo-Duran MD, Martinez-Castillo S, Morales JM, Monleon D, Zanon-Moreno V. A metabolomic approach to dry eye disorders. The role of oral supplements with antioxidants and omega 3 fatty acids. *Mol Vis.* 2015;21:555-567.

335. Tellez-Vazquez J. Omega-3 fatty acid supplementation improves dry eye symptoms in patients with glaucoma: results of a prospective multicenter study. *Clin Ophthalmol.* 2016;10:617-626.

336. Bhargava R, Chandra M, Bansal U, Singh D, Ranjan S, Sharma S. A Randomized Controlled Trial of Omega 3 Fatty Acids in Rosacea Patients with Dry Eye Symptoms. *Curr Eye Res.* Apr 6 2016:1-7.

337. Tanaka H, Harauma A, Takimoto M, Moriguchi T. Association between very long chain fatty acids in the meibomian gland and dry eye resulting from n-3 fatty acid deficiency. *Prostaglandins Leukot Essent Fatty Acids.* Jun 2015;97:1-6.

338. Bhargava R, Kumar P, Phogat H, Kaur A, Kumar M. Oral omega-3 fatty acids treatment in computer vision syndrome related dry eye. *Cont Lens Anterior Eye.* Jun 2015;38(3):206-210.

339. Bhargava R, Kumar P, Arora Y. Short-Term Omega 3 Fatty Acids Treatment for Dry Eye in Young and Middle-Aged Visual Display Terminal Users. *Eye Contact Lens.* Aug 28 2015.

340. Bhargava R, Kumar P. Oral omega-3 fatty acid treatment for dry eye in contact lens wearers. *Cornea.* Apr 2015;34(4):413-420.

341. Liu A, Ji J. Omega-3 essential fatty acids therapy for dry eye syndrome: a meta-analysis of randomized controlled studies. *Med Sci Monit.* 2014;20:1583-1589.

342. Li Z, Choi JH, Oh HJ, Park SH, Lee JB, Yoon KC. Effects of eye drops containing a mixture of omega-3 essential fatty acids and hyaluronic acid on the ocular surface in desiccating stress-induced murine dry eye. *Curr Eye Res.* Sep 2014;39(9):871-878.

343. Hom MM, Asbell P, Barry B. Omegas and Dry Eye: More Knowledge, More Questions. *Optom Vis Sci.* Sep 2015;92(9):948-956.

344. Olenik A, Jimenez-Alfaro I, Alejandre-Alba N, Mahillo-Fernandez I. A randomized, double-masked study to evaluate the effect of omega-3 fatty acids supplementation in meibomian gland dysfunction. *Clin Interv Aging.* 2013;8:1133-1138.

345. Kawakita T, Kawabata F, Tsuji T, Kawashima M, Shimmura S, Tsubota K. Effects of dietary supplementation with fish oil on dry eye syndrome subjects: randomized controlled trial. *Biomed Res.* 2013;34(5):215-220.

346. Bhargava R, Kumar P, Kumar M, Mehra N, Mishra A. A randomized controlled trial of omega-3 fatty acids in dry eye syndrome. *Int J Ophthalmol.* 2013;6(6):811-816.

347. O'Brien TP. The role of bacteria in blepharitis. *Ocul Surf.* Apr 2009;7(2 Suppl):S21-22.

348. Dougherty JM, McCulley JP. Bacterial lipases and chronic blepharitis. *Invest Ophthalmol Vis Sci.* Apr 1986;27(4):486-491.

349. Pflugfelder SC, Karpecki PM, Perez VL. Treatment of blepharitis: recent clinical trials. *Ocul Surf.* Oct 2014;12(4):273-284.

350. Liu J, Sheha H, Tseng SC. Pathogenic role of Demodex mites in blepharitis. *Curr Opin Allergy Clin Immunol.* Oct 2010;10(5):505-510.

351. Gao YY, Di Pascuale MA, Li W, et al. High prevalence of Demodex in eyelashes with cylindrical dandruff. *Invest Ophthalmol Vis Sci.* Sep 2005;46(9):3089-3094.

352. O'Reilly N, Bergin D, Reeves EP, McElvaney NG, Kavanagh K. Demodex-associated bacterial proteins induce neutrophil activation. *Br J Dermatol.* Apr 2012;166(4):753-760.

353. Kim JH, Chun YS, Kim JC. Clinical and immunological responses in ocular demodecosis. *J Korean Med Sci.* Sep 2011;26(9):1231-1237.

354. Jackson WB. Blepharitis: current strategies for diagnosis and management. *Can J Ophthalmol.* Apr 2008;43(2):170-179.

355. Guillon M, Maissa C, Wong S. Eyelid margin modification associated with eyelid hygiene in anterior blepharitis and meibomian gland dysfunction. *Eye Contact Lens.* Sep 2012;38(5):319-325.

356. Guillon M, Maissa C, Wong S. Symptomatic relief associated with eyelid hygiene in anterior blepharitis and MGD. *Eye Contact Lens.* Sep 2012;38(5):306-312.

357. Chronister DR, Kowalski RP, Mah FS, Thompson PP. An independent in vitro comparison of povidone iodine and SteriLid. *J Ocul Pharmacol Ther.* Jun 2010;26(3):277-280.

358. NovaBay Pharmaceuticals. Avenova | Daily Eyelid Cleanser. 2016; http://doctors.avenova.com/. Accessed 24 May 2016, 2016.

359. Connor CG, Choat C, Narayanan S, Kyser K, Rosenberg B, Mulder D. Clinical Effectiveness of Lid Debridement with BlephEx Treatment. *Invest Ophthalmol Vis Sci.* 2015;56(7):4440-4440.

References from Rationale

1. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):75-92.

2. Smith JA, Albeitz J, Begley C, et al. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):93-107.

3. Na KS, Han K, Park YG, Na C, Joo CK. Depression, Stress, Quality of Life, and Dry Eye Disease in Korean Women: A Population-Based Study. *Cornea.* Jul 2015;34(7):733-738.

4. Zeev MS, Miller DD, Latkany R. Diagnosis of dry eye disease and emerging technologies. *Clin Ophthalmol.* 2014;8:581-590.

5. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1938-1978.

6. Ngo W, Srinivasan S, Jones L. Historical overview of imaging the meibomian glands. *J Optom.* 2013;6(1):1-8.

7. Pult H, Nichols JJ. A review of meibography. *Optom Vis Sci.* May 2012;89(5):E760-769.

8. Pult H, Riede-Pult B. Grading Scales in Meibography. *Optom Vis Sci.* 2015;92:E-abstract 150008.

9. OCULUS Inc. The OCULUS Keratograph 5M Technical Data. 2016; http://www.oculus.de/en/products/topography/keratograph-5m/technical-data/. Accessed 14 May 2016, 2016.

10. Arita R. Validity of noninvasive meibography systems: noncontact meibography equipped with a slit-lamp and a mobile pen-shaped meibograph. *Cornea.* Nov 2013;32 Suppl 1:S65-70.

11. Pult H, Riede-Pult BH. Non-contact meibography: keep it simple but effective. *Cont Lens Anterior Eye.* Apr 2012;35(2):77-80.

12. Korb DR, Herman JP, Blackie CA, et al. Prevalence of lid wiper epitheliopathy in subjects with dry eye signs and symptoms. *Cornea.* Apr 2010;29(4):377-383.

13. Yamaguchi M, Kutsuna M, Uno T, Zheng X, Kodama T, Ohashi Y. Marx line: fluorescein staining line on the inner lid as indicator of meibomian gland function. *Am J Ophthalmol.* Apr 2006;141(4):669-675.

14. Korb DR, Herman JP, Greiner JV, et al. Lid wiper epitheliopathy and dry eye symptoms. *Eye Contact Lens.* Jan 2005;31(1):2-8.

15. Blackie CA, Solomon JD, Greiner JV, Holmes M, Korb DR. Inner eyelid surface temperature as a function of warm compress methodology. *Optom Vis Sci.* Aug 2008;85(8):675-683.

16. Lacroix Z, Leger S, Bitton E. Ex vivo heat retention of different eyelid warming masks. *Cont Lens Anterior Eye.* Feb 27 2015.

17. The Eyebag Company Ltd. The EyeBag. 2014; Available at: http://www.eyebagcompany.com/products/The-EyeBag. Accessed December 29, 2015.

18. Bilkhu PS, Naroo SA, Wolffsohn JS. Randomised masked clinical trial of the MGDRx EyeBag for the treatment of meibomian gland dysfunction-related evaporative dry eye. *Br J Ophthalmol.* Dec 2014;98(12):1707-1711.

19. Korb DR, Blackie CA. Debridement-scaling: a new procedure that increases Meibomian gland function and reduces dry eye symptoms. *Cornea.* Dec 2013;32(12):1554-1557.

20. Simmons PA, Liu H, Carlisle-Wilcox C, Vehige JG. Efficacy and safety of two new formulations of artificial tears in subjects with dry eye disease: a 3-month, multicenter, active-controlled, randomized trial. *Clin Ophthalmol.* 2015;9:665-675.

21. Guillon M, Maissa C, Wong S. Symptomatic relief associated with eyelid hygiene in anterior blepharitis and MGD. *Eye Contact Lens.* Sep 2012;38(5):306-312.

22. Bhargava R, Chandra M, Bansal U, Singh D, Ranjan S, Sharma S. A Randomized Controlled Trial of Omega 3 Fatty Acids in Rosacea Patients with Dry Eye Symptoms. *Curr Eye Res.* Apr 6 2016:1-7.

23. Bhargava R, Kumar P, Arora Y. Short-Term Omega 3 Fatty Acids Treatment for Dry Eye in Young and Middle-Aged Visual Display Terminal Users. *Eye Contact Lens.* Aug 28 2015.

References from Imaging Meibomian Glands using Optical Coherence and Confocal Microscopy

1. Mathers WD, Lane JA. Meibomian gland lipids, evaporation, and tear film stability. *Adv Exp Med Biol.* 1998;438:349-360.

2. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1938-1978.

3. Bhamla MS, Chai C, Rabiah NI, Frostad JM, Fuller GG. Instability and Breakup of Model Tear Films. *Invest Ophthalmol Vis Sci.* Mar 1 2016;57(3):949-958.

4. Millar TJ, Schuett BS. The real reason for having a meibomian lipid layer covering the outer surface of the tear film - A review. *Exp Eye Res.* Aug 2015;137:125-138.

5. Schaumberg DA, 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.* Mar 2011;52(4):1994-2005.

6. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1930-1937.

7. Zhang J, Begley CG, Thibos LN, Situ P, Simpson TL, Wu Z. Visual Disturbance and Ocular Irritation in an Experimentally Induced Tear Film Instability Model. *Invest Ophthalmol Vis Sci.* 2014;55(13):1989-1989.

8. Ngo W, Srinivasan S, Jones L. Historical overview of imaging the meibomian glands. *J Optom.* 2013;6(1):1-8.

9. Pult H, Nichols JJ. A review of meibography. *Optom Vis Sci.* May 2012;89(5):E760-769.

10. Tapie R. Etude biomicroscopique des glandes de meibomius. *Annales d'Oculistique.* 1977;210(9):637-648.

11. Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology.* May 2008;115(5):911-915.

12. OCULUS Inc. The OCULUS Keratograph 5M Technical Data. 2016; http://www.oculus.de/en/products/topography/keratograph-5m/technical-data/. Accessed 14 May 2016, 2016.

13. Wei Q, Le Q, Hong J, Xiang J, Wei A, Xu J. In vivo confocal microscopy of meibomian glands and palpebral conjunctiva in vernal keratoconjunctivitis. *Indian J Ophthalmol.* Apr 2015;63(4):327-330.

14. Ibrahim OM, Matsumoto Y, Dogru M, et al. In vivo confocal microscopy evaluation of meibomian gland dysfunction in atopic-keratoconjunctivitis patients. *Ophthalmology.* Oct 2012;119(10):1961-1968.

15. Wang Y, Le Q, Zhao F, et al. Application of in vivo laser scanning confocal microscopy for evaluation of ocular surface diseases: lessons learned from pterygium, meibomian gland disease, and chemical burns. *Cornea.* Oct 2011;30 Suppl 1:S25-28.

16. Matsumoto Y, Sato EA, Ibrahim OM, Dogru M, Tsubota K. The application of in vivo laser confocal microscopy to the diagnosis and evaluation of meibomian gland dysfunction. *Mol Vis.* 2008;14:1263-1271.

17. Fasanella V, Agnifili L, Mastropasqua R, et al. In Vivo Laser Scanning Confocal Microscopy of Human Meibomian Glands in Aging and Ocular Surface Diseases. *Biomed Res Int.* 2016;2016:7432131.

18. Villani E, Canton V, Magnani F, Viola F, Nucci P, Ratiglia R. The aging Meibomian gland: an in vivo confocal study. *Invest Ophthalmol Vis Sci.* Jul 2013;54(7):4735-4740.

19. Agnifili L, Fasanella V, Costagliola C, et al. In vivo confocal microscopy of meibomian glands in glaucoma. *Br J Ophthalmol.* Mar 2013;97(3):343-349.

20. Villani E, Beretta S, De Capitani M, Galimberti D, Viola F, Ratiglia R. In vivo confocal microscopy of meibomian glands in Sjogren's syndrome. *Invest Ophthalmol Vis Sci.* Feb 2011;52(2):933-939.

21. Villani E, Ceresara G, Beretta S, Magnani F, Viola F, Ratiglia R. In vivo confocal microscopy of meibomian glands in contact lens wearers. *Invest Ophthalmol Vis Sci.* Jul 2011;52(8):5215-5219.

22. Kobayashi A, Yoshita T, Sugiyama K. In vivo findings of the bulbar/palpebral conjunctiva and presumed meibomian glands by laser scanning confocal microscopy. *Cornea.* Nov 2005;24(8):985-988.

23. Matsumoto Y, Shigeno Y, Sato EA, et al. The evaluation of the treatment response in obstructive meibomian gland disease by in vivo laser confocal microscopy. *Graefes Arch Clin Exp Ophthalmol.* Jun 2009;247(6):821-829.

24. Bizheva K, Lee P, Sorbara L, Hutchings N, Simpson T. In vivo volumetric imaging of the human upper eyelid with ultrahigh-resolution optical coherence tomography. *J Biomed Opt.* Jul-Aug 2010;15(4):040508.

25. Hwang HS, Park CW, Joo CK. Novel noncontact meibography with anterior segment optical coherence tomography: Hosik meibography. *Cornea.* Jan 2013;32(1):40-43.

26. Hwang HS, Shin JG, Lee BH, Eom TJ, Joo CK. In Vivo 3D Meibography of the Human Eyelid Using Real Time Imaging Fourier-Domain OCT. *PLoS One.* 2013;8(6):e67143.

27. Ju MJ, Shin JG, Hoshi S, et al. Three-dimensional volumetric human meibomian gland investigation using polarization-sensitive optical coherence tomography. *J Biomed Opt.* Mar 2014;19(3):30503.

28. Liang Q, Pan Z, Zhou M, et al. Evaluation of Optical Coherence Tomography Meibography in Patients With Obstructive Meibomian Gland Dysfunction. *Cornea.* Oct 2015;34(10):1193-1199.

29. Tavakoli M, Hossain P, Malik RA. Clinical applications of corneal confocal microscopy. *Clin Ophthalmol.* Jun 2008;2(2):435-445.

30. Al-Mujaini A, Wali UK, Azeem S. Optical coherence tomography: clinical applications in medical practice. *Oman Med J.* Mar 2013;28(2):86-91.

31. Fujimoto JG, Pitris C, Boppart SA, Brezinski ME. Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy. *Neoplasia.* Jan-Apr 2000;2(1-2):9-25.

32. Drexler W, G. FJ. *Optical Coherence Tomography: Technology and Applications*. Berlin: Springer-Verlag; 2008.

33. Que SK, Fraga-Braghiroli N, Grant-Kels JM, Rabinovitz HS, Oliviero M, Scope A. Through the looking glass: Basics and principles of reflectance confocal microscopy. *J Am Acad Dermatol.* Aug 2015;73(2):276-284.

34. Schmitt JM, Knuttel A, Yadlowsky M. Confocal microscopy in turbid media. *J Opt Soc Am A Opt Image Sci Vis.* Aug 1994;11(8):2226-2235.

35. Otchere H. *Use of OCT and Oculus Pentacam HR as Aids to Semi-Scleral Contact Lens Fitting*. Waterloo: Science, University of Waterloo; 2013.

36. Schulze M, Simpson T, Situ P, Menzies K, Walther H, Jones L. Effects of Magnification on Tear Meniscus Parameters Using Optical Coherence Tomography (OCT) Images. *Optom Vis Sci.* 2011;88:E-abstract 115482.

37. Heidelberg Engineering Inc. HRT Rostock Cornea Module. 2015; http://www.heidelbergengineering.com/us/products/hrt-glaucoma-module/cornea-module/. Accessed May 29, 2015.

38. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* Jul 2012;9(7):671-675.

39. Tseng Q. Template Matching and Slice Alignment--- ImageJ Plugins. 2015; https://sites.google.com/site/qingzongtseng/template-matching-ij-plugin. Accessed 16 May, 2016.

40. Ishida S, Nishizawa N. Quantitative comparison of contrast and imaging depth of ultrahigh-resolution optical coherence tomography images in 800-1700 nm wavelength region. *Biomed Opt Express.* Feb 1 2012;3(2):282-294.

41. Yoo Y-S, Byon YS, Joo C-K, Yoon G. Evaluation for Dropout Lesions on Infrared Meibography Using Optical Coherence Tomography Meibography. Paper presented at: The Association for Research in Vision and Ophthalmology Annual Meeting 2016; Seattle, Washington.

42. Xiong X, Wu T, He S. Physical forces make rete ridges in oral mucosa. *Med Hypotheses.* Nov 2013;81(5):883-886.

43. Knop E, Knop N, Zhivov A, et al. The lid wiper and muco-cutaneous junction anatomy of the human eyelid margins: an in vivo confocal and histological study. *J Anat.* Apr 2011;218(4):449-461.

44. Pellacani G, Scope A, Ferrari B, et al. New insights into nevogenesis: in vivo characterization and follow-up of melanocytic nevi by reflectance confocal microscopy. *J Am Acad Dermatol.* Dec 2009;61(6):1001-1013.

45. Yoshita T, Kobayashi A, Sugiyama K. *A case of meibomian pigmentation probably associated with the use of eyeliner.* Vol 54. Ibaraki, JAPON: Association of Folia Ophthalmologica Japonica; 2003.

References from Repeatability of Grading Meibomian Gland Dropout using Two Infrared Systems

1. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1938-1978.

2. Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res.* Mar 2004;78(3):347-360.

3. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):2006-2049.

4. Schein OD, Munoz B, Tielsch JM, Bandeen-Roche K, West S. Prevalence of dry eye among the elderly. *Am J Ophthalmol.* Dec 1997;124(6):723-728.

5. Jie Y, Xu L, Wu YY, Jonas JB. Prevalence of dry eye among adult Chinese in the Beijing Eye Study. *Eye.* Mar 2009;23(3):688-693.

6. Schaumberg DA, 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.* Mar 2011;52(4):1994-2005.

7. Castillanos E, Torres J, Fernandesz I, et al. Preponderance of Evaporative Over Aqueous Deficient-Type Dry Eye Syndrome in Patients With Chronic Dry Eye-Related Symptoms. *Invest Ophthalmol Vis Sci* 2008;49: E-Abstract 2371.

8. Korb DR, Henriquez AS. Meibomian gland dysfunction and contact lens intolerance. *J Am Optom Assoc.* Mar 1980;51(3):243-251.

9. McCulley JP, Sciallis GF. Meibomian keratoconjunctivitis. *Am J Ophthalmol.* Dec 1977;84(6):788-793.

10. Korb DR, Blackie CA. Meibomian gland diagnostic expressibility: correlation with dry eye symptoms and gland location. *Cornea.* Dec 2008;27(10):1142-1147.

11. Ong BL, Hodson SA, Wigham T, Miller F, Larke JR. Evidence for keratin proteins in normal and abnormal human meibomian fluids. *Current eye research.* Dec 1991;10(12):1113-1119.

12. Isreb MA, Greiner JV, Korb DR, et al. Correlation of lipid layer thickness measurements with fluorescein tear film break-up time and Schirmer's test. *Eye.* Jan 2003;17(1):79-83.

13. Foulks GN. The correlation between the tear film lipid layer and dry eye disease. *Surv Ophthalmol.* Jul-Aug 2007;52(4):369-374.

14. Goto E, Tseng SC. Differentiation of lipid tear deficiency dry eye by kinetic analysis of tear interference images. *Arch Ophthalmol.* Feb 2003;121(2):173-180.

15. Mathers WD, Lane JA. Meibomian gland lipids, evaporation, and tear film stability. *Adv Exp Med Biol.* 1998;438:349-360.

16. Robin JB, Jester JV, Nobe J, Nicolaides N, Smith RE. In vivo transillumination biomicroscopy and photography of meibomian gland dysfunction. A clinical study. *Ophthalmology.* Oct 1985;92(10):1423-1426.

17. Jester JV, Rife L, Nii D, Luttrull JK, Wilson L, Smith RE. In vivo biomicroscopy and photography of meibomian glands in a rabbit model of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci.* May 1982;22(5):660-667.

18. Mathers WD, Daley T, Verdick R. Video imaging of the meibomian gland. *Arch Ophthalmol.* Apr 1994;112(4):448-449.

19. Tapie R. Etude biomicroscopique des glandes de meibomius. *Annales d'Oculistique.* 1977;210(9):637-648.

20. Jester JV, Nicolaides N, Smith RE. Meibomian gland studies: histologic and ultrastructural investigations. *Invest Ophthalmol Vis Sci.* Apr 1981;20(4):537-547.

21. Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology.* May 2008;115(5):911-915.

22. McCulley JP, Shine WE, Aronowicz J, Oral D, Vargas J. Presumed hyposecretory/hyperevaporative KCS: tear characteristics. *Trans Am Ophthalmol Soc.* 2003;101:141-152; discussion 152-144.

23. Pult H, Riede-Pult B. Non-contact meibography in diagnosis and treatment of non-obvious meibomian gland dysfunction. *J Optom.* 2011;5(1):2-5.

24. Srinivasan S, Menzies K, Sorbara L, Jones L. Infrared imaging of meibomian gland structure using a novel keratograph. *Optom Vis Sci.* May 2012;89(5):788-794.

25. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* Jul 2012;9(7):671-675.

26. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics.* Mar 1989;45(1):255-268.

27. McBride G. A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. *NIWA Client Report: HAM2005-062.* 2005.

28. Bailey IL, Bullimore MA, Raasch TW, Taylor HR. Clinical grading and the effects of scaling. *Invest Ophthalmol Vis Sci.* Feb 1991;32(2):422-432.

References from A Comparison of Dry Eye Diagnostic Tests between Symptomatic and Asymptomatic Age-Matched Females

1. Schaumberg DA, 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.* Mar 2011;52(4):1994-2005.

2. Uchino M, Nishiwaki Y, Michikawa T, et al. Prevalence and risk factors of dry eye disease in Japan: Koumi study. *Ophthalmology.* Dec 2011;118(12):2361-2367.

3. Ahn JM, Lee SH, Rim TH, et al. Prevalence of and risk factors associated with dry eye: the Korea National Health and Nutrition Examination Survey 2010-2011. *Am J Ophthalmol.* Dec 2014;158(6):1205-1214 e1207.

4. Liu NN, Liu L, Li J, Sun YZ. Prevalence of and risk factors for dry eye symptom in mainland china: a systematic review and meta-analysis. *J Ophthalmol.* 2014;2014:748654.

5. Chia EM, Mitchell P, Rochtchina E, Lee AJ, Maroun R, Wang JJ. Prevalence and associations of dry eye syndrome in an older population: the Blue Mountains Eye Study. *Clin Experiment Ophthalmol.* Jun 2003;31(3):229-232.

6. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):75-92.

7. Sharma A, Hindman HB. Aging: a predisposition to dry eyes. *J Ophthalmol.* 2014;2014:781683.

8. Schein OD, Munoz B, Tielsch JM, Bandeen-Roche K, West S. Prevalence of dry eye among the elderly. *Am J Ophthalmol.* Dec 1997;124(6):723-728.

9. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol.* Aug 2003;136(2):318-326.

10. Gayton JL. Etiology, prevalence, and treatment of dry eye disease. *Clin Ophthalmol.* 2009;3:405-412.

11. Noecker R. Effects of common ophthalmic preservatives on ocular health. *Adv Ther.* Sep-Oct 2001;18(5):205-215.

12. Noecker R, Miller KV. Benzalkonium chloride in glaucoma medications. *Ocul Surf.* Jul 2011;9(3):159-162.

13. Moy A, McNamara NA, Lin MC. Effects of Isotretinoin on Meibomian Glands. *Optom Vis Sci.* Sep 2015;92(9):925-930.

14. Schargus M, Wolf F, Tony HP, Meyer-Ter-Vehn T, Geerling G. Correlation between tear film osmolarity, dry eye disease, and rheumatoid arthritis. *Cornea.* Dec 2014;33(12):1257-1261.

15. Shiboski SC, Shiboski CH, Criswell L, et al. American College of Rheumatology classification criteria for Sjogren's syndrome: a data-driven, expert consensus approach in the Sjogren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res.* Apr 2012;64(4):475-487.

16. Bron AJ, Abelson MB, Ousler G, et al. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):108-152.

17. Mertzanis P, Abetz L, Rajagopalan K, et al. The relative burden of dry eye in patients' lives: comparisons to a U.S. normative sample. *Invest Ophthalmol Vis Sci.* Jan 2005;46(1):46-50.

18. Yu J, Asche CV, Fairchild CJ. The economic burden of dry eye disease in the United States: a decision tree analysis. *Cornea.* Apr 2011;30(4):379-387.

19. Dundas M, Walker A, Woods RL. Clinical grading of corneal staining of non-contact lens wearers. *Ophthalmic Physiol Opt.* Jan 2001;21(1):30-35.

20. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea.* Oct 2003;22(7):640-650.

21. Sweeney DF, Millar TJ, Raju SR. Tear film stability: a review. *Exp Eye Res.* Dec 2013;117:28-38.

22. Wang J, Palakuru JR, Aquavella JV. Correlations among upper and lower tear menisci, non-invasive tear break-up time and Schirmer's test. *Am J Ophthalmol.* 2008;145(5):795-800.

23. Bron AJ, Benjamin L, Snibson GR. Meibomian gland disease. Classification and grading of lid changes. *Eye.* 1991;5 (Pt 4):395-411.

24. Blackie CA, Korb DR, Knop E, Bedi R, Knop N, Holland EJ. Nonobvious obstructive meibomian gland dysfunction. *Cornea.* Dec 2010;29(12):1333-1345.

25. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* May 2000;118(5):615-621.

26. Schaumberg DA, Gulati A, Mathers WD, et al. Development and validation of a short global dry eye symptom index. *Ocul Surf.* Jan 2007;5(1):50-57.

27. Ngo W, Situ P, Keir N, Korb D, Blackie C, Simpson T. Psychometric properties and validation of the Standard Patient Evaluation of Eye Dryness questionnaire. *Cornea.* Sep 2013;32(9):1204-1210.

28. Bron AJ, Yokoi N, Gaffney EA, Tiffany JM. A solute gradient in the tear meniscus. II. Implications for lid margin disease, including meibomian gland dysfunction. *Ocul Surf.* Apr 2011;9(2):92-97.

29. Bron AJ, Yokoi N, Gaffney EA, Tiffany JM. A solute gradient in the tear meniscus. I. A hypothesis to explain Marx's line. *Ocul Surf.* Apr 2011;9(2):70-91.

30. Doughty MJ, Naase T, Donald C, Hamilton L, Button NF. Visualisation of "Marx's line" along the marginal eyelid conjunctiva of human subjects with lissamine green dye. *Ophthalmic Physiol Opt.* Jan 2004;24(1):1-7.

31. Korb DR, Greiner JV, Herman JP, et al. Lid-wiper epitheliopathy and dry-eye symptoms in contact lens wearers. *CLAO J.* Oct 2002;28(4):211-216.

32. Korb DR, Herman JP, Greiner JV, et al. Lid wiper epitheliopathy and dry eye symptoms. *Eye Contact Lens.* Jan 2005;31(1):2-8.

33. Korb DR, Herman JP, Blackie CA, et al. Prevalence of lid wiper epitheliopathy in subjects with dry eye signs and symptoms. *Cornea.* Apr 2010;29(4):377-383.

34. Varikooty J, Srinivasan S, Subbaraman L, et al. Variations in observable lid wiper epitheliopathy (LWE) staining patterns in wearers of silicone hydrogel lenses. *Cont Lens Anterior Eye.* Dec 2015;38(6):471-476.

35. Korb DR, Blackie CA. Debridement-scaling: a new procedure that increases Meibomian gland function and reduces dry eye symptoms. *Cornea.* Dec 2013;32(12):1554-1557.

36. Doughty MJ. Morphological features of cells along Marx's line of the marginal conjunctiva of the human eyelid. *Clin Exp Optom.* Jan 2013;96(1):76-84.

37. Yamaguchi M, Kutsuna M, Uno T, Zheng X, Kodama T, Ohashi Y. Marx line: fluorescein staining line on the inner lid as indicator of meibomian gland function. *Am J Ophthalmol.* Apr 2006;141(4):669-675.

38. Ngo W, Caffery B, Srinivasan S, Jones LW. Effect of Lid Debridement-Scaling in Sjogren Syndrome Dry Eye. *Optom Vis Sci.* Sep 2015;92(9):e316-320.

39. Knop E, Knop N, Zhivov A, et al. The lid wiper and muco-cutaneous junction anatomy of the human eyelid margins: an in vivo confocal and histological study. *J Anat.* Apr 2011;218(4):449-461.

40. Pult H, Purslow C, Murphy PJ. The relationship between clinical signs and dry eye symptoms. *Eye.* Apr 2011;25(4):502-510.

41. Mathers WD, Shields WJ, Sachdev MS, Petroll WM, Jester JV. Meibomian gland dysfunction in chronic blepharitis. *Cornea.* Jul 1991;10(4):277-285.

42. Kim S, Blackie CA, Korb DR. Age related anteroplacement of the line of marx and contact lens wear. *Optom Vis Sci.* 2012;89:E-abstract 125380.

43. Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology.* May 2008;115(5):911-915.

44. Miller KL, Walt JG, Mink DR, et al. Minimal clinically important difference for the ocular surface disease index. *Arch Ophthalmol.* Jan 2010;128(1):94-101.

45. Amaechi O, Osunwoke C. The relation between invasive and non-invasive tear break-up time in young adults. *Journal of the Nigerian Optometric Association.* 2004;11(1).

46. van Bijsterveld OP. Diagnostic tests in the Sicca syndrome. *Arch Ophthalmol.* Jul 1969;82(1):10-14.

47. OCULUS Inc. The OCULUS Keratograph 5M. 2015; Available at: http://www.oculus.de/us/products/topography/keratograph-5m/highlights/. Accessed March 31, 2015.

48. Li N, Deng XG, He MF. Comparison of the Schirmer I test with and without topical anesthesia for diagnosing dry eye. *Int J Ophthalmol.* Aug 18 2012;5(4):478-481.

49. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. *Cornea.* Nov 2004;23(8):762-770.

50. Sullivan BD, Crews LA, Messmer EM, et al. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications. *Acta Ophthalmol.* Mar 2014;92(2):161-166.

51. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):2006-2049.

52. Millar TJ, Schuett BS. The real reason for having a meibomian lipid layer covering the outer surface of the tear film - A review. *Exp Eye Res.* Aug 2015;137:125-138.

53. Zhang J, Begley CG, Thibos LN, Situ P, Simpson TL, Wu Z. Visual Disturbance and Ocular Irritation in an Experimentally Induced Tear Film Instability Model. *Invest Ophthalmol Vis Sci.* 2014;55(13):1989-1989.

54. Alex A, Edwards A, Hays JD, et al. Factors predicting the ocular surface response to desiccating environmental stress. *Invest Ophthalmol Vis Sci.* May 2013;54(5):3325-3332.

55. Bilkhu PS, Naroo SA, Wolffsohn JS. Randomised masked clinical trial of the MGDRx EyeBag for the treatment of meibomian gland dysfunction-related evaporative dry eye. *Br J Ophthalmol.* Dec 2014;98(12):1707-1711.

56. Friedland BR, Fleming CP, Blackie CA, Korb DR. A novel thermodynamic treatment for meibomian gland dysfunction. *Curr Eye Res.* Feb 2011;36(2):79-87.

57. Arita R, Morishige N, Shirakawa R, Sato Y, Amano S. Effects of Eyelid Warming Devices on Tear Film Parameters in Normal Subjects and Patients with Meibomian Gland Dysfunction. *Ocul Surf.* Oct 2015;13(4):321-330.

58. Borchman D, Foulks GN, Yappert MC, et al. Physical changes in human meibum with age as measured by infrared spectroscopy. *Ophthalmic Res.* 2010;44(1):34-42.

59. Eom Y, Choi KE, Kang SY, Lee HK, Kim HM, Song JS. Comparison of meibomian gland loss and expressed meibum grade between the upper and lower eyelids in patients with obstructive meibomian gland dysfunction. *Cornea.* May 2014;33(5):448-452.

60. Lutz W, Sanderson W, Scherbov S. The coming acceleration of global population ageing. *Nature.* Feb 7 2008;451(7179):716-719.

61. Sriprasert I, Warren DW, Mircheff AK, Stanczyk FZ. Dry eye in postmenopausal women: a hormonal disorder. *Menopause.* Mar 2016;23(3):343-351.

References from The Effect of an Eyelid Warming Device on Meibomian Gland Dysfunction

1. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1938-1978.

2. Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res.* Mar 2004;78(3):347-360.

3. Jester JV, Nicolaides N, Kiss-Palvolgyi I, Smith RE. Meibomian gland dysfunction. II. The role of keratinization in a rabbit model of MGD. *Invest Ophthalmol Vis Sci.* May 1989;30(5):936-945.

4. Jester JV, Rife L, Nii D, Luttrull JK, Wilson L, Smith RE. In vivo biomicroscopy and photography of meibomian glands in a rabbit model of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci.* May 1982;22(5):660-667.

5. Smith JA, Albeitz J, Begley C, et al. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):93-107.

6. Nichols KK, Foulks GN, Bron AJ, et al. The international workshop on meibomian gland dysfunction: executive summary. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1922-1929.

7. Yu J, Asche CV, Fairchild CJ. The economic burden of dry eye disease in the United States: a decision tree analysis. *Cornea.* Apr 2011;30(4):379-387.

8. Mertzanis P, Abetz L, Rajagopalan K, et al. The relative burden of dry eye in patients' lives: comparisons to a U.S. normative sample. *Invest Ophthalmol Vis Sci.* Jan 2005;46(1):46-50.

9. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1930-1937.

10. Schein OD, Munoz B, Tielsch JM, Bandeen-Roche K, West S. Prevalence of dry eye among the elderly. *Am J Ophthalmol.* Dec 1997;124(6):723-728.

11. Jie Y, Xu L, Wu YY, Jonas JB. Prevalence of dry eye among adult Chinese in the Beijing Eye Study. *Eye.* Mar 2009;23(3):688-693.

12. Schaumberg DA, 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.* Mar 2011;52(4):1994-2005.

13. Ding J, Sullivan DA. Aging and dry eye disease. *Exp Gerontol.* Jul 2012;47(7):483-490.

14. Nien CJ, Massei S, Lin G, et al. Effects of age and dysfunction on human meibomian glands. *Arch Ophthalmol.* Apr 2011;129(4):462-469.

15. Moy A, McNamara NA, Lin MC. Effects of Isotretinoin on Meibomian Glands. *Optom Vis Sci.* Sep 2015;92(9):925-930.

16. Ding J, Kam WR, Dieckow J, Sullivan DA. The influence of 13-cis retinoic acid on human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci.* Jun 2013;54(6):4341-4350.

17. Kremer I, Gaton DD, David M, Gaton E, Shapiro A. Toxic effects of systemic retinoids on meibomian glands. *Ophthalmic Res.* 1994;26(2):124-128.

18. Lambert RW, Smith RE. Effects of 13-cis-retinoic acid on the hamster meibomian gland. *J Invest Dermatol.* Mar 1989;92(3):321-325.

19. Sriprasert I, Warren DW, Mircheff AK, Stanczyk FZ. Dry eye in postmenopausal women: a hormonal disorder. *Menopause.* Oct 27 2015.

20. Truong S, Cole N, Stapleton F, Golebiowski B. Sex hormones and the dry eye. *Clin Exp Optom.* Jul 2014;97(4):324-336.

21. Azcarate PM, Venincasa VD, Feuer W, Stanczyk F, Schally AV, Galor A. Androgen deficiency and dry eye syndrome in the aging male. *Invest Ophthalmol Vis Sci.* Aug 2014;55(8):5046-5053.

22. Arita R, Itoh K, Inoue K, Kuchiba A, Yamaguchi T, Amano S. Contact lens wear is associated with decrease of meibomian glands. *Ophthalmology.* Mar 2009;116(3):379-384.

23. Geerling G, Tauber J, Baudouin C, et al. The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):2050-2064.

24. Jackson TL. Moorfields Manual of Ophthalmology. In: Jackson TL, ed. *Second Edition*. London: JP Medical Ltd; 2014.

25. Lacroix Z, Leger S, Bitton E. Ex vivo heat retention of different eyelid warming masks. *Cont Lens Anterior Eye.* Jun 2015;38(3):152-156.

26. Blackie CA, Solomon JD, Greiner JV, Holmes M, Korb DR. Inner eyelid surface temperature as a function of warm compress methodology. *Optom Vis Sci.* Aug 2008;85(8):675-683.

27. Doan S, Chiambaretta F, Baudouin C. Evaluation of an eyelid warming device (Blephasteam) for the management of ocular surface diseases in France: the ESPOIR study. *J Fr Ophtalmol.* Dec 2014;37(10):763-772.

28. Bilkhu PS, Naroo SA, Wolffsohn JS. Randomised masked clinical trial of the MGDRx EyeBag for the treatment of meibomian gland dysfunction-related evaporative dry eye. *Br J Ophthalmol.* Dec 2014;98(12):1707-1711.

29. Finis D, Hayajneh J, Konig C, Borrelli M, Schrader S, Geerling G. Evaluation of an automated thermodynamic treatment (LipiFlow(R)) system for meibomian gland dysfunction: a prospective, randomized, observer-masked trial. *Ocul Surf.* Apr 2014;12(2):146-154.

30. Purslow C. Evaluation of the ocular tolerance of a novel eyelid-warming device used for meibomian gland dysfunction. *Cont Lens Anterior Eye.* Oct 2013;36(5):226-231.

31. The Eyebag Company Ltd. The EyeBag Company. 2014; Available at: http://www.eyebagcompany.com/index.php. Accessed December 29, 2015.

32. The Eyebag Company Ltd. The EyeBag. 2014; Available at: http://www.eyebagcompany.com/products/The-EyeBag. Accessed December 29, 2015.

33. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.* May 2007;39(2):175-191.

34. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods.* Nov 2009;41(4):1149-1160.

35. Friedland BR, Fleming CP, Blackie CA, Korb DR. A novel thermodynamic treatment for meibomian gland dysfunction. *Curr Eye Res.* Feb 2011;36(2):79-87.

36. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* May 2000;118(5):615-621.

37. Schaumberg DA, Gulati A, Mathers WD, et al. Development and validation of a short global dry eye symptom index. *Ocul Surf.* Jan 2007;5(1):50-57.

38. Zhao Y, Tan CL, Tong L. Intra-observer and inter-observer repeatability of ocular surface interferometer in measuring lipid layer thickness. *BMC Ophthalmol.* 2015;15:53.

39. Cho P, Douthwaite W. The relation between invasive and noninvasive tear break-up time. *Optom Vis Sci.* Jan 1995;72(1):17-22.

40. Sorbara L, Peterson R, Schneider S, Woods C. Comparison between live and photographed slit lamp grading of corneal staining. *Optom Vis Sci.* Mar 2015;92(3):312-317.

41. Korb DR, Blackie CA. Meibomian gland diagnostic expressibility: correlation with dry eye symptoms and gland location. *Cornea.* Dec 2008;27(10):1142-1147.

42. Ngo W, Srinivasan S, Schulze M, Jones L. Repeatability of grading meibomian gland dropout using two infrared systems. *Optom Vis Sci.* Jun 2014;91(6):658-667.

43. Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology.* May 2008;115(5):911-915.

44. Situ P, Simpson T, Chalmers R, Wu Z, Begley C. Validation of the Current Symptoms Questionnaire (CSQ): a meta-analytical approach. *Invest Ophthalmol Vis Sci.* 2013;54(15):6023-6023.

45. Situ P, Chalmers R, Wu Z, Simpson T, Begley C. Using Current Symptoms Questionnaire to Evaluate Changes in Symptoms Induced by Different Experimental Conditions. *Optom Vis Sci.* 2013;90:E-Abstract 6023.

46. MetricWire. MetricWire: Mobile Data Collection Made Easy. 2015; Available at: http://www.metricwire.com/. Accessed December 30, 2015.

47. Stewart-Williams S, Podd J. The placebo effect: dissolving the expectancy versus conditioning debate. *Psychol Bull.* Mar 2004;130(2):324-340.

48. Brown MT, Bussell JK. Medication adherence: WHO cares? *Mayo Clin Proc.* Apr 2011;86(4):304-314.

49. Parfitt GJ, Xie Y, Geyfman M, Brown DJ, Jester JV. Absence of ductal hyper-keratinization in mouse age-related meibomian gland dysfunction (ARMGD). *Aging.* Nov 2013;5(11):825-834.

50. McCann LC, Tomlinson A, Pearce EI, Diaper C. Tear and meibomian gland function in blepharitis and normals. *Eye Contact Lens.* Jul 2009;35(4):203-208.

References from The Effect of Lid-Debridement-Scaling in Sjögren's Syndrome Dry Eye

1. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):75-92.

2. Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjogren's syndrome. *Nat Rev Rheumatol.* Sep 2010;6(9):529-537.

3. Shiboski SC, Shiboski CH, Criswell L, et al. American College of Rheumatology classification criteria for Sjogren's syndrome: a data-driven, expert consensus approach in the Sjogren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res.* Apr 2012;64(4):475-487.

4. Shimazaki J, Goto E, Ono M, Shimmura S, Tsubota K. Meibomian gland dysfunction in patients with Sjogren syndrome. *Ophthalmology.* Aug 1998;105(8):1485-1488.

5. Villani E, Beretta S, De Capitani M, Galimberti D, Viola F, Ratiglia R. In vivo confocal microscopy of meibomian glands in Sjogren's syndrome. *Invest Ophthalmol Vis Sci.* Feb 2011;52(2):933-939.

6. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1938-1978.

7. Korb DR, Blackie CA. Debridement-scaling: a new procedure that increases Meibomian gland function and reduces dry eye symptoms. *Cornea.* Dec 2013;32(12):1554-1557.

8. Bron AJ, Yokoi N, Gaffney EA, Tiffany JM. A solute gradient in the tear meniscus. I. A hypothesis to explain Marx's line. *Ocul Surf.* Apr 2011;9(2):70-91.

9. Bron AJ, Yokoi N, Gaffney EA, Tiffany JM. A solute gradient in the tear meniscus. II. Implications for lid margin disease, including meibomian gland dysfunction. *Ocul Surf.* Apr 2011;9(2):92-97.

10. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* May 2000;118(5):615-621.

11. Schaumberg DA, Gulati A, Mathers WD, et al. Development and validation of a short global dry eye symptom index. *Ocul Surf.* Jan 2007;5(1):50-57.

12. Whitcher JP, Shiboski CH, Shiboski SC, et al. A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjogren's Syndrome International Registry. *Am J Ophthalmol.* Mar 2010;149(3):405-415.

13. Friedland BR, Fleming CP, Blackie CA, Korb DR. A novel thermodynamic treatment for meibomian gland dysfunction. *Curr Eye Res.* Feb 2011;36(2):79-87.

14. Kim S, Blackie CA, Korb DR. Age related anteroplacement of the line of marx and contact lens wear. *Optom Vis Sci.* 2012;89:E-abstract 125380.

15. Villani E, Canton V, Magnani F, Viola F, Nucci P, Ratiglia R. The aging Meibomian gland: an in vivo confocal study. *Invest Ophthalmol Vis Sci.* Jul 2013;54(7):4735-4740.

16. Butovich IA, Wojtowicz JC, Molai M. Human tear film and meibum. Very long chain wax esters and (O-acyl)-omega-hydroxy fatty acids of meibum. *J Lipid Res.* Dec 2009;50(12):2471-2485.

17. Ashraf Z, Pasha U, Greenstone V, et al. Quantification of human sebum on skin and human meibum on the eye lid margin using Sebutape(R), spectroscopy and chemical analysis. *Curr Eye Res.* Jun 2011;36(6):553-562.

18. Pult H, Purslow C, Murphy PJ. The relationship between clinical signs and dry eye symptoms. *Eye.* Apr 2011;25(4):502-510.

19. Sullivan BD, Crews LA, Messmer EM, et al. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications. *Acta Ophthalmol.* Mar 2014;92(2):161-166.

20. Miller KL, Walt JG, Mink DR, et al. Minimal clinically important difference for the ocular surface disease index. *Arch Ophthalmol.* Jan 2010;128(1):94-101.

21. Johnson ME. The association between symptoms of discomfort and signs in dry eye. *Ocul Surf.* Oct 2009;7(4):199-211.

22. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. *Cornea.* Nov 2004;23(8):762-770.

23. Roethlisberger FJ, Dickson WJ. *Management and the Worker*. Cambridge: Harvard University Press; 1939.

References from The Relief of Dry Eye Signs and Symptoms Using a Combination of Lubricants, Lid Hygiene, and Ocular Nutraceuticals

1. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):75-92.

2. Miljanovic B, Dana R, Sullivan DA, Schaumberg DA. Impact of dry eye syndrome on vision-related quality of life. *Am J Ophthalmol.* Mar 2007;143(3):409-415.

3. Friedman NJ. Impact of dry eye disease and treatment on quality of life. *Curr Opin Ophthalmol.* Jul 2010;21(4):310-316.

4. Li M, Gong L, Chapin WJ, Zhu M. Assessment of vision-related quality of life in dry eye patients. *Invest Ophthalmol Vis Sci.* Aug 2012;53(9):5722-5727.

5. Bron AJ, Abelson MB, Ousler G, et al. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):108-152.

6. Schaumberg DA, Gulati A, Mathers WD, et al. Development and validation of a short global dry eye symptom index. *Ocul Surf.* Jan 2007;5(1):50-57.

7. Ngo W, Situ P, Keir N, Korb D, Blackie C, Simpson T. Psychometric properties and validation of the Standard Patient Evaluation of Eye Dryness questionnaire. *Cornea.* Sep 2013;32(9):1204-1210.

8. Dougherty BE, Nichols JJ, Nichols KK. Rasch analysis of the Ocular Surface Disease Index (OSDI). *Invest Ophthalmol Vis Sci.* Nov 2011;52(12):8630-8635.

9. Grubbs J, Jr., Huynh K, Tolleson-Rinehart S, et al. Instrument development of the UNC Dry Eye Management Scale. *Cornea.* Nov 2014;33(11):1186-1192.

10. Pflugfelder SC, Geerling G, Kinoshita S, et al. Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf.* Apr 2007;5(2):163-178.

11. Geerling G, Tauber J, Baudouin C, et al. The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):2050-2064.

12. Guillon M, Maissa C, Wong S. Symptomatic relief associated with eyelid hygiene in anterior blepharitis and MGD. *Eye & contact lens.* Sep 2012;38(5):306-312.

13. Chronister DR, Kowalski RP, Mah FS, Thompson PP. An independent in vitro comparison of povidone iodine and SteriLid. *J Ocul Pharmacol Ther.* Jun 2010;26(3):277-280.

14. Epstein A, Pang L, Najafi-Tagol K, Najafi R, Stroman D, Debabov D. Comparison of Bacterial Lipase Activity in the Presence of Eye Lid Cleansers. *Invest Ophthalmol Vis Sci.* 2015;56(7):4446-4446.

15. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Archives of ophthalmology.* May 2000;118(5):615-621.

16. Sullivan BD, Whitmer D, Nichols KK, et al. An objective approach to dry eye disease severity. *Invest Ophthalmol Vis Sci.* Dec 2010;51(12):6125-6130.

17. Finis D, Pischel N, Schrader S, Geerling G. Evaluation of lipid layer thickness measurement of the tear film as a diagnostic tool for Meibomian gland dysfunction. *Cornea.* Dec 2013;32(12):1549-1553.

18. Baek J, Doh SH, Chung SK. Comparison of Tear Meniscus Height Measurements Obtained With the Keratograph and Fourier Domain Optical Coherence Tomography in Dry Eye. *Cornea.* Oct 2015;34(10):1209-1213.

19. Amaechi O, Osunwoke C. The relation between invasive and non-invasive tear break-up time in young adults. *Journal of the Nigerian Optometric Association.* 2004;11(1).

20. Sorbara L, Peterson R, Schneider S, Woods C. Comparison between live and photographed slit lamp grading of corneal staining. *Optom Vis Sci.* Mar 2015;92(3):312-317.

21. Korb DR, Herman JP, Blackie CA, et al. Prevalence of lid wiper epitheliopathy in subjects with dry eye signs and symptoms. *Cornea.* Apr 2010;29(4):377-383.

22. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea.* Nov 2003;22(7):640-650.

23. Bron AJ, Benjamin L, Snibson GR. Meibomian gland disease. Classification and grading of lid changes. *Eye.* 1991;5 (Pt 4):395-411.

24. Ngo W, Srinivasan S, Schulze M, Jones L. Repeatability of grading meibomian gland dropout using two infrared systems. *Optom Vision Sci.* Jun 2014;91(6):658-667.

25. Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology.* May 2008;115(5):911-915.

26. Ong NH, Purcell TL, Roch-Levecq AC, et al. Epithelial Healing and Visual Outcomes of Patients Using Omega-3 Oral Nutritional Supplements Before and After Photorefractive Keratectomy: A Pilot Study. *Cornea.* Jun 2013;32(6):761-765.

27. Wojtowicz JC, Butovich I, Uchiyama E, Aronowicz J, Agee S, McCulley JP. Pilot, prospective, randomized, double-masked, placebo-controlled clinical trial of an omega-3 supplement for dry eye. *Cornea.* Mar 2011;30(3):308-314.

28. Macsai MS. The role of omega-3 dietary supplementation in blepharitis and meibomian gland dysfunction (an AOS thesis). *Trans Am Ophthalmol Soc.* 2008;106:336-356.

29. Kangari H, Eftekhari MH, Sardari S, et al. Short-term consumption of oral omega-3 and dry eye syndrome. *Ophthalmology.* Nov 2013;120(11):2191-2196.

30. Simmons PA, Liu H, Carlisle-Wilcox C, Vehige JG. Efficacy and safety of two new formulations of artificial tears in subjects with dry eye disease: a 3-month, multicenter, active-controlled, randomized trial. *Clin Ophthalmol.* 2015;9:665-675.

31. Simmons PA, Carlisle-Wilcox C, Vehige JG. Comparison of novel lipid-based eye drops with aqueous eye drops for dry eye: a multicenter, randomized controlled trial. *Clin Ophthalmol.* 2015;9:657-664.

32. Rangel-Huerta OD, Aguilera CM, Mesa MD, Gil A. Omega-3 long-chain polyunsaturated fatty acids supplementation on inflammatory biomakers: a systematic review of randomised clinical trials. *Br J Nutr.* Jun 2012;107 Suppl 2:S159-170.

33. Olenik A, Jimenez-Alfaro I, Alejandre-Alba N, Mahillo-Fernandez I. A randomized, double-masked study to evaluate the effect of omega-3 fatty acids supplementation in meibomian gland dysfunction. *Clin Interv Aging.* 2013;8:1133-1138.

34. Stahl U, Willcox M, Stapleton F. Osmolality and tear film dynamics. *Clin Exp Optom.* Jan 2012;95(1):3-11.

35. Bunya VY, Fuerst NM, Pistilli M, et al. Variability of Tear Osmolarity in Patients With Dry Eye. *JAMA Ophthalmol.* Jun 2015;133(6):662-667.

References from General Discussion and Future Work

1. Tapie R. Etude biomicroscopique des glandes de meibomius. *Annales d'Oculistique.* 1977;210(9):637-648.

2. Hwang HS, Shin JG, Lee BH, Eom TJ, Joo CK. In Vivo 3D Meibography of the Human Eyelid Using Real Time Imaging Fourier-Domain OCT. *PLoS One.* 2013;8(6):e67143.

3. Hwang HS, Park CW, Joo CK. Novel noncontact meibography with anterior segment optical coherence tomography: Hosik meibography. *Cornea.* Jan 2013;32(1):40-43.

4. Liang Q, Pan Z, Zhou M, et al. Evaluation of Optical Coherence Tomography Meibography in Patients With Obstructive Meibomian Gland Dysfunction. *Cornea.* Oct 2015;34(10):1193-1199.

5. Ju MJ, Shin JG, Hoshi S, et al. Three-dimensional volumetric human meibomian gland investigation using polarization-sensitive optical coherence tomography. *J Biomed Opt.* Mar 2014;19(3):30503.

6. Helmchen F, Denk W. Deep tissue two-photon microscopy. *Nat Methods.* Dec 2005;2(12):932-940.

7. Bailey IL, Bullimore MA, Raasch TW, Taylor HR. Clinical grading and the effects of scaling. *Invest Ophthalmol Vis Sci.* Feb 1991;32(2):422-432.

8. Millar TJ, Schuett BS. The real reason for having a meibomian lipid layer covering the outer surface of the tear film - A review. *Exp Eye Res.* Aug 2015;137:125-138.

9. Alex A, Edwards A, Hays JD, et al. Factors predicting the ocular surface response to desiccating environmental stress. *Invest Ophthalmol Vis Sci.* May 2013;54(5):3325-3332.

10. Zhang J, Begley CG, Thibos LN, Situ P, Simpson TL, Wu Z. Visual Disturbance and Ocular Irritation in an Experimentally Induced Tear Film Instability Model. *Invest Ophthalmol Vis Sci.* 2014;55(13):1989-1989.

11. Blackie CA, Solomon JD, Greiner JV, Holmes M, Korb DR. Inner eyelid surface temperature as a function of warm compress methodology. *Optom Vis Sci.* Aug 2008;85(8):675-683.

12. Lacroix Z, Leger S, Bitton E. Ex vivo heat retention of different eyelid warming masks. *Cont Lens Anterior Eye.* Feb 27 2015.

13. Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res.* Mar 2004;78(3):347-360.

14. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):1938-1978.

15. Korb DR, Blackie CA. Debridement-scaling: a new procedure that increases Meibomian gland function and reduces dry eye symptoms. *Cornea.* Dec 2013;32(12):1554-1557.

16. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Invest Ophthalmol Vis Sci.* Mar 2011;52(4):2006-2049.

17. Sook Chun Y, Park IK. Reliability of 4 clinical grading systems for corneal staining. *Am J Ophthalmol.* May 2014;157(5):1097-1102.

18. Akpek EK, Smith RA. Overview of age-related ocular conditions. *Am J Manag Care.* May 2013;19(5 Suppl):S67-75.

19. Kumar N, Feuer W, Lanza NL, Galor A. Seasonal Variation in Dry Eye. *Ophthalmology.* Aug 2015;122(8):1727-1729.

20. van Tilborg M, Kort H, Murphy P, Evans K. The influence of dry eye and office environment on visual functioning. *Stud Health Technol Inform.* 2015;217:427-431.

21. Sirois FM. Motivations for consulting complementary and alternative medicine practitioners: a comparison of consumers from 1997-8 and 2005. *BMC Complement Altern Med.* 2008;8:16.

22. Lin T, Gong L, Liu X, Ma X. Fourier-domain optical coherence tomography for monitoring the lower tear meniscus in dry eye after acupuncture treatment. *Evid Based Complement Alternat Med.* 2015;2015:492150.

23. Yang L, Yang Z, Yu H, Song H. Acupuncture therapy is more effective than artificial tears for dry eye syndrome: evidence based on a meta-analysis. *Evid Based Complement Alternat Med.* 2015;2015:143858.

24. Sano K, Kawashima M, Ikeura K, Arita R, Tsubota K. Abdominal breathing increases tear secretion in healthy women. *Ocul Surf.* Jan 2015;13(1):82-87.

25. Frass M, Strassl RP, Friehs H, Mullner M, Kundi M, Kaye AD. Use and acceptance of complementary and alternative medicine among the general population and medical personnel: a systematic review. *Ochsner J.* Spring 2012;12(1):45-56.

Appendices

Appendices from Imaging Meibomian Glands using Optical Coherence Tomography and Confocal Microscopy

Appendix 1:

Experimental spectral domain OCT specifications:

Spectral domain OCT with ~6μm axial resolution (bandwidth 50nm centered around 840nm)

Ultra-long scan depth ~7.2mm

High speed 24k A-line per second

Width scan = up to 18mm in 3D

Computer controlled fixation target

Autofocusing colour camera viewing system with low illumination

X-Y alignment for scan positioning

Compact power supply for galvanometer

Manual adjustment of focal plane

All-in-one OCT software

The settings used to capture the meibomian glands were:

Integration time	72
Line #	12000
Scan orientation	X-scan
Y-ON	YES
Nominal scan width	4.96
Nominal scan height	4.93
Scan type	3D
Focal plane at	1.0
Scan pattern	2048 32 x 4mm

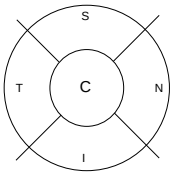
Appendix 2:

Specifications for the HRT3/RCM[37]

Focus range	Max 1500μm
Image size	400 μm x 400 μm
Resolution (transversal)	~1 μm/ pixel
Digital image size	384 x 384 pixels
Microscope lens	63x
Light source	670 nm wavelength
Image acquisition time	0.024 sec per 2D image
CCD camera image	640 x 480 pixels
Acquisition modes	Section, volume (40 images over 80 μm depth), movie sequence

Appendices from Repeatability of Grading Meibomian Gland Dropout using Two Infrared Systems

Appendix 1: Summary of clinical tests and grading scales

Clinical tests	Grading scale
Corneal Staining	 Type: 0 – 100 0: no staining 25: micropunctate 50: macropunctate 75: coalescence 100: patch Extent: 0 – 100 Number represents total area of staining in that zone (S = superior, T = temporal, N = nasal, I = inferior) Depth: 0 – 4 0: none 1: epithelial 2: stromal (delayed) 3: stromal (confined) 4: stromal (diffuse) Zone score = Type*Extent*Depth Total score = sum of zone score in each zone
Telangiectasia	0: none 1: one single telangiectasia 2: 2-5 telangiectasia 3: >5 telangiectasia 4: entire lid involvement
MG orifice obstruction using digital expression of ~1.5g over the inferior central 8 orifices	0: no orifices contain turbid secretions 1: less than 1/3 of orifices, but at least one contain turbid secretions 2: between 1/3 and 2/3 of orifices contain turbid secretions 3: more than 2/3 of orifices contain turbid secretions 4: all orifices plugged with turbid secretions
Vascularity	0: none 1: minimal 2: mild 3: moderate 4: severe
Lash loss	0: none 1: minimal 2: mild 3: moderate 4: severe

Appendix 2: Raw grading scores for both observers on both days

Image Number	Day 1		Day 2	
	Observer 1	Observer 2	Observer 1	Observer 2
1	2	2	1	0
2	0	1	1	2
3	1	1	1	1
4	0	0	0	0
5	0	1	0	0
6	0	1	0	1
7	0	1	1	1
8	1	2	2	2
9	0	0	0	0
10	3	2	3	2
11	2	3	0	0
12	1	1	2	1
13	1	2	1	2
14	0	0	1	1
15	0	0	1	1
16	1	0	0	0
17	0	0	0	0
18	0	1	1	1
19	0	0	0	0
20	1	1	2	0
21	0	0	0	0
22	1	1	1	1
23	1	2	1	1
24	1	0	0	0
25	0	1	0	0
26	1	0	0	0
27	1	1	0	1
28	1	0	1	1
29	1	0	1	1

30	3	3	3	3
31	1	1	0	0
32	1	2	1	1
33	0	1	1	1
34	1	1	1	1
35	3	3	3	3
36	1	1	1	1
37	2	1	1	1
38	0	0	0	0
39	2	2	1	2
40	0	0	0	0
41	0	0	0	0
42	0	0	1	2
43	0	0	0	0
44	0	0	0	0
45	0	0	0	0
46	0	0	0	0
47	1	1	1	0
48	0	0	1	1
49	1	0	1	0
50	3	2	3	2
51	1	1	1	1
52	1	0	1	1
53	0	0	0	0
54	0	0	0	0
55	0	0	0	0
56	1	0	1	0
57	1	1	1	1
58	0	0	0	0
59	1	1	1	1
60	1	0	0	0
61	0	0	0	0

62	0	0	0	0
63	1	0	1	0
64	0	0	0	0
65	0	0	1	0
66	0	0	0	0
67	1	1	1	0
68	0	0	0	0
69	0	0	0	0
70	3	3	3	3
71	1	1	0	0
72	0	0	0	0
73	1	2	1	2
74	0	0	0	0
75	1	1	1	1
76	0	0	0	0
77	2	2	2	1
78	1	2	0	0
79	2	2	2	2
80	0	0	0	0
81	1	1	1	2
82	1	1	0	0
83	1	1	1	1
84	0	0	1	0
85	0	1	0	0
86	0	0	1	0
87	1	0	1	1
88	1	1	2	1
89	0	0	0	0
90	3	3	3	3
91	1	0	0	0
92	1	0	1	1
93	2	2	1	0

94	0	0	0	0
95	0	1	0	0
96	0	0	0	0
97	0	1	0	1
98	1	1	1	0
99	1	0	1	1
100	0	0	0	0
101	0	0	0	0
102	0	0	0	0
103	2	1	2	1
104	0	0	1	0
105	1	1	2	1
106	0	1	0	0
107	1	0	1	0
108	0	0	0	0
109	2	1	1	1
110	3	3	3	3
111	1	0	1	0
112	1	1	1	1
113	1	1	1	1
114	1	1	1	1
115	3	3	3	3
116	1	1	1	1
117	1	1	0	1
118	0	0	0	0
119	1	2	1	1
120	1	0	0	0
121	1	0	1	0
122	2	2	2	2
123	0	0	0	0
124	0	0	0	0
125	0	0	0	0

126	0	0	0	0
127	1	1	1	1
128	2	1	1	1
129	0	0	1	0
130	3	2	2	2
131	1	1	1	1
132	1	1	1	1
133	0	0	0	0
134	0	0	0	0
135	0	0	0	0
136	0	0	1	0
137	1	1	1	1
138	1	1	0	0
139	1	0	1	1
140	1	1	1	0
141	1	0	1	1
142	0	0	0	0
143	1	0	1	0
144	1	1	1	0
145	0	0	0	0
146	1	1	1	1
147	0	0	0	0
148	0	0	0	0
149	0	0	0	0
150	3	3	3	3
151	1	1	0	0
152	1	0	0	0
153	2	1	1	1
154	0	0	0	0
155	1	1	1	1
156	0	0	0	0
157	2	1	2	1

158	1	1	0	0
159	2	2	2	2
160	0	0	0	0

Appendices from A Comparison of Dry Eye Diagnostic Tests between Symptomatic and Asymptomatic Age-Matched Females

Table A1: Meibum quality grading scale

Grade	Meibum quality
0	Normal, clear, may have a few particles
1	Opaque with normal viscosity
2	Opaque with increased viscosity
3	Severe thickening (toothpaste)

Table A2: Eyelid margin score derivation

Eyelid Margin Feature	Grade
Erythema (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4=severe)	0 1 2 3 4
Lash Loss (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4= severe)	0 1 2 3 4
Edema of lid margin (0 = absent, 1 = present)	0 1
Lid Margin Telangiectasia (0 = none; 1 = single telangiectasia; 2 = 2 to 5 telangiectasia; 3 = > 5 telangiectasia; 4=severe-entire lid involvement)	0 1 2 3 4

Table A3: Line of Marx grading scale

Grade	Clinical feature of the Line of Marx
0	Mostly (>75%) posterior to the orifices
1	Mostly bisecting the orifices
1	Mixed posterior and bisecting the orifices
2	Mostly anterior
2	Mixed posterior, bisecting and anterior to the orifices
2	Mixed bisecting and anterior to the orifices

Table A4: Lid wiper epitheliopathy grading scale

Horizontal length of staining	Grade	Sagittal width of staining	Grade
<2mm	0	<25% of the width of wiper	0
2-4mm	1	25% - <50% width of wiper	1
5-9mm	2	50% - <75% width of wiper	2
>10mm	3	≥ 75% of the width of wiper	3

Fluorescein Grade = (Horizontal + Sagittal) / 2

Lissamine Green Grade = (Horizontal + Sagittal) / 2

Final LWE Grade = (Fluorescein Grade + Lissamine Green Grade) / 2

Table A5: Meibomian gland dropout grading scale

Grade	Meibomian gland dropout
0	No dropout
1	Between 0 to 1/3 of the lid
2	1/3 to 2/3 of the entire lid
3	More than 2/3 of the entire lid

Table A6: Means and standard deviations for the symptomatic and asymptomatic groups for future sample size determination.

	Symptomatic Group	Asymptomatic Group
Age	59.4 ± 8.5	59.8 ± 8.7
OSDI (0-100)	40.0 ± 20.4	3.6 ± 4.0
Visual Acuity (logMAR)	0.02 ± 0.10	0.03 ± 0.11
Ocular staining (0-12)	5.2 ± 2.1	1.1 ± 1.8
Meibum quality (0-3)	2.8 ± 0.4	2.2 ± 0.7
Number of glands obstructed (0-8)	6.6 ± 1.8	4.2 ± 2.6
NIBUT (seconds)	2.4 ± 1.3	3.9 ± 2.3
Eyelid margin score (0-13)	6.8 ± 2.3	7.2 ± 2.7
Meiboscore (0-6)	2.5 ± 1.8	1.7 ± 0.9
Lid wiper epitheliopathy (0-3)	0.52 ± 0.81	0.42 ± 0.85
Marx's line placement (0-2)	0.85 ± 0.88	0.40 ± 0.68
Schirmer's Test (0-30)	11.5 ± 8.5	11.9 ± 11.0

Appendices from The Effect of an Eyelid Warming Device on Meibomian Gland Dysfunction

Temperature curve of the EyeBag after heating

An EyeBag was heated in 900W microwave (RCA, USA) for 30 seconds according to manufacturer instructions.[32] After removal from the microwave, the EyeBag was lightly shaken to evenly distribute the heated seeds. The silver side (silk) was set down flat against a wooden surface and centered on top of a HH-20A series digital thermometer probe (Omega Engineering, Stamford, Connecticut, USA). The temperature of the EyeBag was recorded every 5 seconds for the first 5 minutes and every 30 seconds for the subsequent 5 minutes, for a total duration of 10 minutes. This was repeated two more times and the mean temperature for each time point was recorded. This procedure was repeated with another 2 separate EyeBags subsequently (within 5 minutes).

After removal from the microwave, the temperatures of the EyeBags continued to slowly climb. The peak temperature of each EyeBag occurred at approximately 3 minutes before slowly decaying over the course of 10 minutes. The maximum for EyeBag 1, 2 and 3 was 38.7°C @ 4:35 mins, 40.4°C @ 2:45 mins, and 39.2°C @ 3:25 mins, respectively. Mean temperatures for EyeBag 1, 2, and 3 at the end of the 10 minute period was 38.2°C, 38.9°C, and 38.2°C respectively.

A two-way ANOVA with post-hoc Tukey's multiple comparison test was used to determine the statistical significance of the difference between the EyeBags at different time points.

Prior to microwaving (t = Pre), there was no significant difference between all 3 EyeBags. However, EyeBag 1 and EyeBag 2 were significantly different between t = 5s and t = 80s inclusive, with maximum difference of 3.3°C occurring at the t = 20s mark. EyeBags 2 and 3 were not significantly different from each other at all time points. EyeBags 1 and 3 were not significantly different from each other at all time points. Figure 6-4 is a temperature-time curve highlighting the temperature retention of each EyeBag.

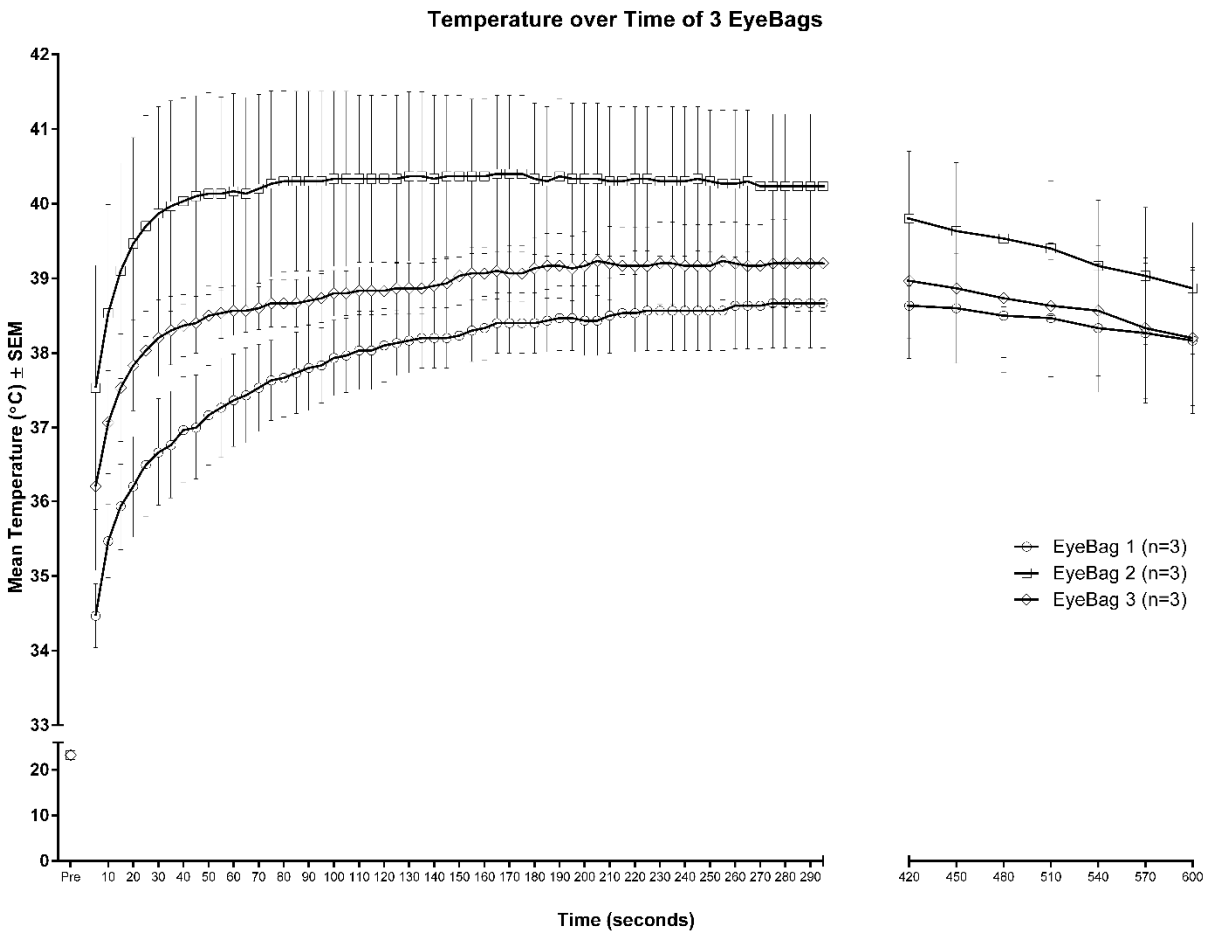


Figure 6-4: Mean temperature-over-time curves of 3 different EyeBags. Temperatures continued to quickly rise for the first 60s before slowly plateauing and decaying slowly. At the end of the 600s duration, the temperature of all EyeBags were at least 38.1°C. EyeBag 3 was not significantly different than EyeBags 1 or 2 at all time-points. EyeBag 1 and 2 was significantly different only between t=5s and t=80s.

Appendices from The Relief of Dry Eye Signs and Symptoms Using a Combination of Lubricants, Lid Hygiene, and Ocular Nutraceuticals

Table A.1: Product information sheet (all Advanced Vision Research, Inc.)

Products	**TheraTears® Lubricant Eye Drop (15mL)**	**TheraTears® Nutrition (90 pack)**	**TheraTears® SteriLid Eyelid Cleanser (48mL)**
Dosage	Ophthalmic, 1 or 2 drops, prn	Oral, 3 capsules QD	Ophthalmic, 1 or 2 application OU, QD
Active Ingredients	0.25% sodium carboxymethylcellulose	1 serving = 3 softgels Per serving: Omega-3 fatty acids: EPA 450mg DHA 300mg Flaxseed Oil (organic): 1000mg	N/A
Inactive Ingredients	Sodium chloride, potassium chloride, sodium bicarbonate, calcium chloride, magnesium chloride, sodium phosphate, borate buffers, Dequest and purified water; sodium perborate	Gelatin, glycerin USP, Vitamin E (in soybean oil), purified water USP	Water, PEG 80, Sorbitan Laurate, Sodium Trideceth Sulfate, Cocamidopropyl Betaine, Sodium Lauroamphoacetate, PEG 150 Distearate, Sodium Laureth 13 Carboxylate, Linalool Oil, Hepes Acetate, Sodium Perborate Monohydrate, Panthenol, Allantoin (Comfrey Root), Sodium Chloride, Tea Tree (Melaleuca Alternifolia) Oil, Tris EDTA, Boric Acid, Cocamidopropyl PG Dimonium Chloride, Etridronic Acid, Citric Acid for pH adjustment, Sodium Hydroxide for pH adjustment
Preservative	Sodium perborate	None	Sodium Perborate , EDTA

Table A.2: Components of the eyelid margin score

Eyelid Margin Feature	**Grade**
Erythema (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4=severe)	0 1 2 3 4
Lash Loss (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4= severe)	0 1 2 3 4
Edema of lid margin (0 = absent, 1 = present)	0 1
Lid Margin Telangiectasia (0 = none; 1 = single telangiectasia; 2 = 2 to 5 telangiectasia; 3 = > 5 telangiectasia; 4=severe-entire lid involvement)	0 1 2 3 4

Table A.3: Grading components for LWE

Horizontal length of staining	Grade	Sagittal width of staining	Grade
<2mm	0	<25% of the width of wiper	0
2-4mm	1	25% - <50% width of wiper	1
5-9mm	2	50% - <75% width of wiper	2
>10mm	3	≥ 75% of the width of wiper	3

Fluorescein Grade = (Horizontal + Sagittal) / 2

Lissamine Green Grade = (Horizontal + Sagittal) / 2

Final LWE Grade = (Fluorescein Grade + Lissamine Green Grade) / 2