Novel methods for the evaluation of the tear film in the diagnosis of dry eye

by

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Author’s declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Dry eye is a complex, multi-factorial disease that results in a compromised tear film and ocular surface. Clinicians and researchers alike have historically relied on an individual's symptoms to diagnose and manage the condition, due to a lack of reliable objective methods for quantifying disease presence and severity. Of late, parameters such as tear film osmolarity and tear meniscus height have shown promise as valid methods for enumerating characteristics of the tear film that may aid the diagnosis of dry eye.

Two new technologies have recently been introduced that can measure said parameters. The TearLab™ is a novel handheld nano-osmometer capable of measuring tear film osmolarity on samples as small as 50 nL. The device uses electrical conductance to measure osmolarity, and the small sample requirements purportedly allows the device to minimally disturb the natural state of the tear film. The RTVue-100 is a spectral-, or Fourier-domain optical coherence tomographer that has the ability to generate high resolution, cross-sectional images of the tear meniscus, and subsequently measure tear meniscus height. As little is published on the use of these technologies to evaluate the tear film, a series of studies was completed to determine their performance in both a normal and dry eye population.

Chapter 2:

Aim: To compare anterior segment spectral-domain OCT (SOCT) tear meniscus height measures to those from the more commonly used time-domain OCT (TOCT).

Methods: The right eye of 50 healthy subjects had images of their TMH captured with TOCT (OCT2, Carl Zeiss Meditec, Dublin, CA, USA) and SOCT (RTVue-100, Optovue, Freemont, CA, USA). Data were acquired using two different anterior segment lenses, the CAM-S and CAM-L on the SOCT. Images were then analyzed for differences in their derived TMH.

Results: The average TMH for TOCT was 0.280 ± 0.139mm, while the mean TMH measured using the SOCT was 0.354 ± 0.163mm and 0.345 ± 0.167mm for the CAM-S and CAM-L respectively. There was a significant difference ($p < 0.001$) when comparing TOCT to either of the SOCT lenses. There was no statistically significant difference between the CAM-L and CAM-S ($p=1.0$). Bland-Altman analysis showed poor agreement between TOCT and SOCT (95% limits of agreement -0.138 to +0.285mm for the CAM-S and -0.185 to +0.315mm for the CAM-L).

Conclusion: The RTVue-100 produces TMH measurements that are significantly higher than OCT2 in a normal patient population. However, the RTVue-100 showed a number of other advantages over the OCT2 in the measurement and analysis of images. Future work needs to determine the causative factors behind the observed differences.

Chapter 3:

Aim: To generate data on the performance of the TearLab™ nano-osmometer in a control and dry eye population. Of particular interest is which time interval between successive tear collections, 15 minutes or 1 minute, yields the most repeatable result in normals and those with dry eye. A secondary outcome is the determination of the effect, if any, that tear collection using glass capillary tubes has on osmolarity of the sample.

Methods: This was a two-phase study that recruited 20 subjects (10 normals / 10 dry eye) for participation in phase I and 30 subjects (15 normals / 15 dry eye) for participation in phase II. As part of phase I, subjects had eight tear collections performed on each eye at each of three visits, four separated by 15 minutes and four separated by 1 minute. Phase II consisted of four visits, each visit involving the collection of four tear samples using the TearLab™, and one collection of 5 µL using a glass capillary tube. Tear break-up times (TBUT) were recorded at the end of each visit for both phases.

Results: During phase I, mean osmolarity in the control group was 287.9 ± 7.5 mOsm/L and for the dry eye group, mean osmolarity was 297.8 ± 14.7 mOsm/L, a difference that was statistically significant ($p=0.007$). Significant differences were also observed between groups for OSDI ($p<0.001$) and TBUT ($p=0.029$). No significant difference was observed whether collections were performed at 15 minutes or 1 minute, and intraclass correlation coefficients were 0.899 and 0.923, respectively. A bilinear function fit to osmolarity and TBUT data showed a strong relationship between the two variables. During phase II, mean osmolarity was 287.7 ± 8.01 mOsm/L in the control group and 295.8 ± 12.5 mOsm/L in the dry eye group ($p = 0.0058$). When using the glass capillary tube, mean osmolarity dropped to 282.4 ± 9.8 mOsm/L in the control group and 291.1 ± 9.9 mOsm/L in the dry eye group. Comparisons between measures taken directly with the TearLab™ and those with the capillary tube were significantly different in the control group ($p = 0.006$), but were not significantly different in the dry eye group ($p = 0.10$).

Conclusion: No change in osmolarity was observed whether tears were collected in rapid succession or given time to equilibrate. To improve collection efficiency, a 60-90 second time interval between consecutive tear collections using the TearLab™ is advocated to avoid perturbation of the tear film's natural state. Tear collection using a glass capillary tube appears to induce a reflex tearing response, which results in an artificial lowering of tear osmolarity. The relationship observed between tear osmolarity and TBUT suggests that those suffering from dry eye have an inherent tear film instability.

Chapter 4:

Aim: To use *in vitro* methods to determine the effect of different TearLab™ chip card production lots on osmolarity, and the effect of glass capillary tube collection on osmolarity.

Methods: Three different chip card lots (Code 3, 5 and 10) were used to measure osmolarity of two control solutions with known osmotic concentration: 292 mOsm/L and 338 mOsm/L. Six measures were taken on each control solution with each chip card lot. The same two control solutions, in

addition to a contrived artificial tear solution with an osmolarity of 294 mOsm/L, were also measured directly with the TearLab™ or glass capillary tube. 18 measures were obtained on each solution using each method, for a total of 108 measures.

Results: Regardless of control osmolarity, batch 10 was significantly different than batch 3 or 5 ($p < 0.002$), while the mean osmolarity of batch 3 and 5 were statistically similar ($p > 0.78$). Mean osmolarity of those samples taken directly from the vial was 295.8 ± 19.22 mOsm/L, and 300.3 ± 18.9 mOsm/L for those that were first collected via capillary tube, a difference which was not statistically significant ($p = 0.23$).

Conclusion: Different TearLab™ chip card production lots can have an effect on the measured tear osmolarity, and therefore caution must be used when comparing measures over time. *In vitro* use of the glass capillary tube to collect fluid samples does not significantly influence osmolarity values.

Chapter 5:

Aim: To determine if any relationship exists between tear osmolarity measures and tear meniscus height, and whether symptoms of dry eye correlate with either variable.

Methods: 45 subjects completed linear visual analogue scales (VAS) that graded five symptoms of dry eye (comfort, dryness, burning, grittiness and clarity of their vision). This was followed by three measures of the lower tear meniscus height using the RTVue-100, and four measures of tear osmolarity using the TearLab™, both procedures being performed on each eye. Subjects were grouped into one of four categories based on a combination of their mean osmolarity and TMH, using 300 mOsm/L and 0.3mm as a cut-off respectively.

Results: Mean TMH for the right eye was 0.281 ± 0.123mm and for the left eye was 0.297 ± 0.130mm, a difference that was not statistically significant ($p = 0.34$). Osmolarity values for the right and left eye, 302.9 ± 16.4 mOsm/L and 301.2 ± 14.8 mOsm/L respectively, were also not significantly different ($p = 0.21$) and Pearson's correlation coefficient failed to establish a significant

relationship between TMH and osmolarity ($r = -0.14$, $p = 0.36$). No difference was observed between each of the five symptoms rated as part of the linear VAS ($p = 0.99$), and no relationship between any of the symptoms and signs could be established regardless of which group was evaluated.

Conclusion: There does not appear to be a relationship between osmolarity and TMH, and no predominant symptoms could be identified within distinct dry eye groups. Nonetheless, the present study has made some interesting observations regarding the possible development of dry eye and its sub-types with increasing age.

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Chapter 1
Introduction

1.1 The normal tear film

Historically, the tear film has been thought of as a trilaminar fluid, with an outermost lipid layer, a thick central aqueous layer, and a pre-epithelial mucus layer.[1] In recent years this model has evolved, and the tear film is now described as an aqueous gel gradient with an overlying lipid layer.[2] This inner aqueous layer consists of a dense mucin-phase closest to the ocular surface, and a decreasing density of mucins as one moves toward the lipid layer. Each component of the tear film has a distinct origin and purpose.

The aqueous portion is primarily produced by the lacrimal and accessory lacrimal glands, namely the glands of Wolfring and Krause. There are numerous constituents that make up this layer, including water, oxygen, proteins, electrolytes, growth factors, peptides and inflammatory mediators. The predominant electrolytes in the tear film are sodium, potassium, magnesium, calcium, chloride, and bicarbonate, and phosphate ions are found within the tear film aqueous layer. These electrolytes serve to maintain the pH of the tear film (normally 7.2 – 7.6)[3] and also play a role in determining its osmolarity.[4] The major proteins of the aqueous play an integral role in defence of the ocular surface. Among these proteins are lysozyme (an enzyme that attacks the peptidoglycan cell walls of gram-negative bacteria and buffers the tear film),[5] lipocalin (a lipid scavenging protein that plays a role in forming the surface tension of the tears and has also been shown to have endonuclease function),[6, 7] lactoferrin (which has anti-microbial activity via its ability to sequester iron ions, in addition to anti-inflammatory effects, and promotes cell growth and DNA synthesis)[8] and secretory IgA (an antibody that plays a role in mucosal immunity).[9] These four proteins each count for 15-20% of the total protein contained within the tear film.[10]

Approximately 20 ocular mucins have been identified and they can be divided into two classes: secreted and soluble.[11] The secreted ones are typically membrane-bound to the cells that produce them, the corneal and conjunctival epithelia. They form the glycocalyx, which is a gelatinous layer of secreted mucins that provides a lubricating layer, and ensures an even spread of the tear film over the hydrophobic epithelial cells.[12] Other research has shown the glycocalyx to have an antimicrobial effect, as it prevents adherence of bacteria, viruses and inflammatory cells to the ocular surface.[13] The soluble mucins, most notably MUC 5AC secreted by the conjunctival goblet cells, interact with the membrane-bound mucins and the aqueous layer to form a gel that traps water.[14] This ensures that the mucin gel remains hydrated and can function to protect and maintain the epithelium from environmental insults.

The lipid layer is secreted by the meibomian glands and functions to eliminate evaporation of the aqueous layer and facilitate the tear film spreading over the corneal surface. Lipids are only found on the anterior surface of the tear film, and this is thought to be because any free lipids circulating in the aqueous are immediately bound to lipocalin.[15] The meibomian lipid is made up of phospholipids, free fatty acids and cholesterol.[16]

The lacrimal glands, meibomian glands and goblet cells are collectively integrated into a system known as the Lacrimal Functional Unit (LFU). The LFU also includes the cornea, conjunctiva, and sensory and motor nerves of the ocular surface, and is chiefly responsible for the health of the ocular surface via the production of tears.[17] Basal tear production is primarily controlled by constant stimulation of the trigeminal sensory fibres, which passes through to the pterygo-palatine ganglion to post-ganglionic fibres that terminate in the lacrimal and meibomian glands and goblet cells.[17] Reflex tears are a response to external physical or chemical stimuli of the trigeminal nerve, and follow the same afferent and efferent pathway as basal tears.[18] A third type of tears, emotional,

are generated in higher centres of the brain and may be a response to a variety of emotions, including fear, sadness, and anger, among others.[19]

1.2 Purpose of the tear film

The tear film has three primary functions: creation of a high quality refracting interface, maintenance of epithelial cell health, and protection of the ocular surface. The tear film-cornea combination has been shown to provide the eye with 80% of the eye's refractive power.[20] Therefore, a smooth, even tear film provides optimal vision, and any deviations in tear film stability will result in increased aberrations and decreased visual acuity.[21] In addition to a smooth refracting surface, a requirement of ideal vision is a cornea that is transparent. It achieves this is by being a virtually avascular tissue, which creates a problem for the corneal epithelial cells to receive the necessary nutrients to continue to function. This issue is overcome by the tear film, which constantly bathes the ocular surface and creates the required trophic environment. Limbal vessels are too far away to supply the oxygen demands of the entire cornea, so the tear film is called upon to act as an intermediary between the oxygenated air and the corneal epithelium. It also contains approximately 25µg/mL of glucose,[22] providing a secondary source of glucose in addition to that supplied by the aqueous humor. Finally, it contains a number of growth factors, anti-oxidants and peptides that encourage wound healing and corneal regeneration.[10] One of the most important purposes the tear film serves is to protect the ocular surface from insult, both physical and antimicrobial. It provides physical protection by washing away harmful contaminants such as allergens, bacteria, or airborne pollutants through the process of reflex tearing, in conjunction with the blink reaction. The mucous layer serves as a physical barrier to prevent bacteria from accessing the corneal epithelium by trapping, adsorbing and removing them from the ocular surface.[23] It also acts as a lubricant to reduce the shear forces exerted on the eye during each blink.[11] Antimicrobial protection is provided via two mechanisms, one being the active enzymes lysozyme and lactoferrin (among others) outlined

previously, and the other is a complex, immunologically-derived response from lymphocytes and cytokines such as IL-1, TNF and TGF-β.[23]

A breakdown in function of any of the layers or constituents of the tear film can result in destabilization, which over time may affect the ocular surface and ultimately result in what is known as dry eye.

1.3 What is dry eye?

In 2006, the Dry Eye Workshop (DEWS) convened a sub-committee of leading experts on dry eye with the stated goal of redefining the term dry eye.[24] Using the definition that came out of the 1995 National Eye Institute/Industry Dry Eye Workshop as a framework,[25] the sub-committee integrated new knowledge regarding the core mechanisms and visual consequences of dry eye to come up with the following definition:

> '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.'[24]

As shown in Figure 1-1, a complex cycle is behind the development of dry eye, and it was the opinion of the committee that the core mechanisms are tear hyperosmolarity and tear film instability. Tear hyperosmolarity activates a cascade of signalling molecules within the surface epithelial cells, which leads to the release of inflammatory mediators at the ocular surface.[26-28] Epithelial injury causes epithelial damage, a loss of goblet cells, and a disturbance of mucin expression leading to tear film instability.[29-30] This instability leads to tear-break up and hyperosmolarity, creating a vicious circle of events. The aetiology of dry eye is generally broken down into two classes: aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE). It is important to note that these two classes are not mutually exclusive, and the existence of one does not preclude the existence of the other.

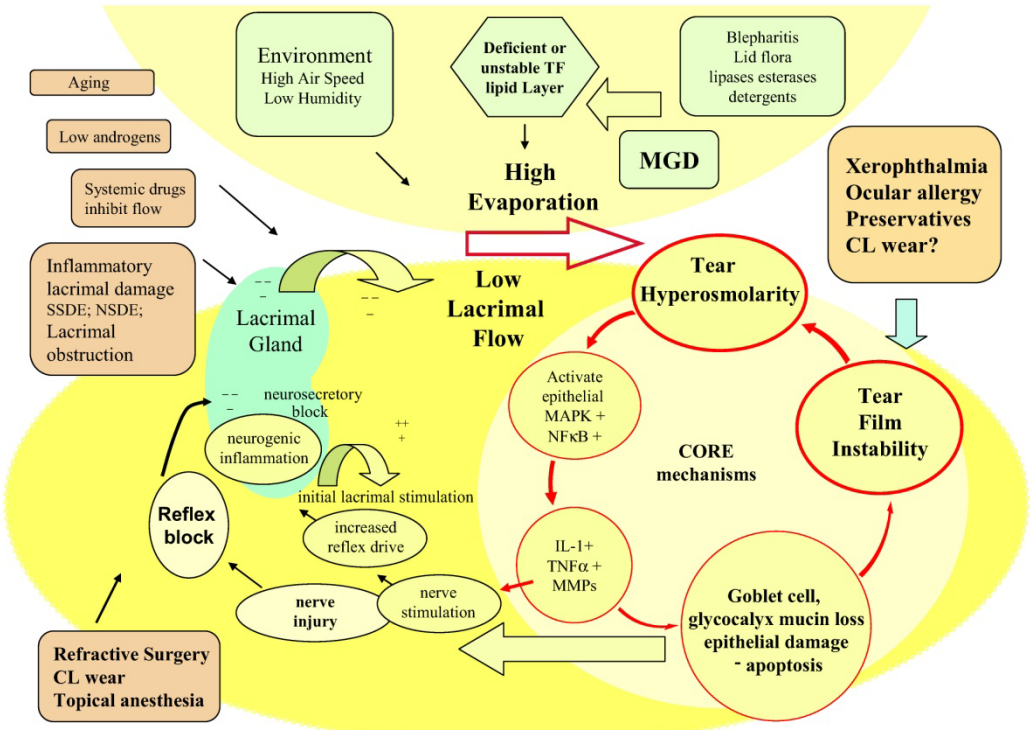

Figure 1-1. Mechanism of dry eye as hypothesized by the sub-committee on the definition of dry eye as part of the Dry Eye Workshop (reproduced with permission from *Ocular Surface* 2007. 5(2):75-92).

ADDE is primarily attributed to reduced secretion of the aqueous layer due to lacrimal dysfunction, most commonly the result of an inflammatory reaction.[31] ADDE can be loosely sub-divided into two categories: Sjögren Syndrome dry eye and non- Sjögren Syndrome dry eye. Sjögren syndrome dry eye is a devastating chronic autoimmune disorder of the exocrine glands with associated lymphocytic infiltration of the lacrimal gland that impairs its proper functioning.[32] Non-Sjögren syndrome dry eye is usually the result of lacrimal deficiency, or lacrimal duct blockage, although systemic medications have been shown to be a factor.[33] In ADDE, it is hypothesized that the tear film becomes hyperosmolar due to the low volume of tears present on the ocular surface, in combination with a normal to increased rate of evaporation.[34] EDE is a result of increased evaporation of the tears from the ocular surface in the presence of a normally-functioning lacrimal gland,

ultimately increasing the osmolarity of the tear film.[24] Its causes have been sub-divided into intrinsic (causes which are the result of lid dysfunction, most commonly meibomian gland dysfunction) or extrinsic (external causes, including contact lens wear and allergy). The effects of EDE may not be as apparent in the initial stages of the disease, as a reflex compensatory response of the lacrimal gland can help in maintaining a normal osmolarity. However, excessive reflex stimulation of the lacrimal gland can induce an inflammatory response within the gland, leading to the release of inflammatory mediators into the tears and exacerbating the symptoms of dry eye.[35]

The prevalence of dry eye in the United States varies widely, and has been reported to be between 7.8%[36] and 14.6%[37] in large population-based studies. Incidence of dry eye has been evaluated using data from the Beaver Dam study, and was reported as 21.6% over a 10-year period.[38] In Europe, prevalence has been estimated to be <0.1%,[39] while in Japan, Shimura used questionnaires to determine a prevalence of 33%.[40] Closer to home, the CANDEES (Canadian Dry Eye Epidemiology Study) study noted a prevalence of approximately 1 in 4 among patients presenting for routine optometric care.[41] The reason for the variation in prevalence and incidence estimates depends on countless factors, including the population characteristics, definition of dry eye used to make the diagnosis, and perhaps most importantly, the tests used to make the diagnosis. Many large-scale epidemiological studies use questionnaires in lieu of objective tests, which can be an issue due to the poor correlation between signs and symptoms, and also because of the tendency of those who are ill to respond to questionnaires, over-representing the condition.[41-43]

The social and economic impact of dry eye cannot be overstated. Those suffering from dry eye are significantly more likely than those without dry eye to report problems reading, performing professional work, and driving during the day or night.[44] Schiffman et al. did a utility assessment evaluated by the time-trade off method and found that those with moderate-to-severe dry eye had similar utility scores to moderate angina, highlighting how significantly dry eye impacts an

individual's life.[45] The economic impact is difficult to assess, as the costs may be direct (office visits, medication), indirect (lost workplace productivity, sick days) and intangible (decreased quality of life).[46] One study estimated that monthly out of pocket costs are $25 in a non-Sjögren Syndrome dry eye population.[47] The same study showed that dry eye interfered with work an average of 184 days per year, which the authors estimated to be a productivity loss of >$5000 per patient per year.[47] Older estimates have placed the cost of artificial tears for the relief/treatment of dry eye within the US to be $100 million dollars,[48] an estimate that is likely much higher now considering the significant increase in the population over age 65 in North America.[49-50]

A secondary goal of the DEWS report was to come up with a globally agreed upon criterion for the screening, diagnosis and monitoring of dry eye .[51] The aim was to use single tests or a combination of tests that could be performed in a clinical environment in a cost-effective manner, with appropriate sensitivity and specificity. It was also imperative that accepted cut-offs be derived for tests to aid in the appropriate discrimination of those affected and unaffected with the disease. They ultimately agreed that some of the most important existing and emerging technologies for the screening and diagnosis of dry eye included: symptom assessment through the use of questionnaires, the assessment of tear stability using tear break-up times, the measurement of tear osmolarity, and the measurement of tear meniscus height as an estimate of tear volume.[51]

1.4 Questionnaires

Many would argue that dry eye is a symptom-driven disease, and questionnaires are a simple and effective means of screening individuals for its presence. Some of the most commonly used validated questionnaires include the McMonnies dry eye history questionnaire,[52-54] the Ocular Surface Disease Index (OSDI),[55-56] and the Dry Eye Questionnaire (DEQ).[57-58] Common features among the four questionnaires include an evaluation of the frequency and intensity of symptoms, effect of symptoms on daily function and vision, and the effect of environment, among others.[43] Linear visual

analogue scales have also been employed with success, although this is not a validated method of symptom evaluation.[59]

McMonnies was one of the first to develop a comprehensive dry eye questionnaire based on an evaluation of available literature at the time.[52] The result was a 14-item questionnaire primarily consisting of questions with yes/no answers, and some questions dedicated to the elucidation of various risk factors, including contact-lens wear, medications, gender and age. An evaluation of symptoms (soreness, scratchiness, dryness, grittiness, burning) was also included. A scoring index for the questionnaire allows possible scores to be between 0 and 45, a higher score presumed to correlate with increased severity. The sensitivity and specificity of the questionnaire for the diagnosis of dry eye when a cut-off of >14.5 is used has been reported to be as high as 98% and 97% respectively;[54, 60] however, a psychometric validation study of the questionnaire determined that using a cut-off score of >14.5 yielded a sensitivity of 82% and specificity of 36%.[61] The questionnaire also demonstrates poor internal reliability, suggesting its use as an indicator of treatment success may be limited.[61]

The OSDI is a twelve item questionnaire that uses a Likert scale to evaluate the frequency of symptoms over a 1 week period.[55] Factor analysis revealed that there are 3 sub-scales within the OSDI: vision-related function (6 questions), ocular symptoms (3 questions) and environmental triggers (3 questions).[56] Possible scores of the OSDI range between 0 and 100, and as with the McMonnies questionnaire, a greater score represents greater disability. A cut-off of 17 has been suggested to maximize sensitivity and specificity of the test at differentiating between normals and those with severe dry eye, although scoring is usually broken down into 0-12 (normal), 13-22 (mild), 23-32 (moderate) and >33 (severe).[56] Internal reliability and test-retest repeatability of the OSDI is markedly higher than the McMonnies,[56] making it a popular test for use in clinical trials.[62-66] However, to use the OSDI to chart treatment efficiency, it is important to know the minimal clinically important difference (MCID), which is the smallest difference in test score that would mandate a

change in a patient's management. Miller determined that the MCID for mild-moderate dry eye is 4.5 – 7.3, and the MCID for severe disease is 7.3 – 13.4.[67] This can be interpreted as meaning that an improvement in a mild-moderate subject's OSDI score of 4.5 represents a true improvement in symptoms.

The DEQ is a 21-item questionnaire developed by Begley et al. that evaluates risk factors in addition to the frequency of specific symptoms.[57] A five-question subset of this questionnaire was recently validated, the five questions pertaining to the frequency with which eye discomfort, dryness and watering occurred, and the end-of-day severity of the discomfort and dryness.[68] It was found that this simple five item questionnaire could discriminate between those with and without dry eye, with a sensitivity of 90% and specificity of 81% using a score of ≥ 6.[68] Work has been done comparing the above three questionnaires to one another using Spearmann correlations, which found significant correlations between the overall scores.[69] The authors suggested that the three questionnaires are measuring the same thing (ocular dryness symptoms) based on their unidimensionality, and therefore advocated using only one of the questionnaires at a given time.[69]

Despite being a non-validated measure, linear analogue scales have demonstrated utility in giving additional insight into the severity of various symptoms of dry eye. These are simple scales from 0 – 100, with 100 representing the absence of the symptom, and 0 representing a severe manifestation of the symptom. The primary disadvantage of these scales is their non-linearity and large response range, which can make analysis and interpretation of the results difficult.[70]

1.5 Tear stability

A stable tear film is a necessary component of a healthy ocular surface, and a requirement for clear vision.[21, 71-72] As mentioned earlier, deviations in the constituents and composition of the tear film can lead to instability, jeopardizing vision and the comfort and health of the ocular surface. Its recognition in the DEWS report as a core mechanism in dry eye has made the dynamics of tear film

instability an area of great interest; however, the physical phenomenon itself is still poorly understood. Mechanisms that have been proposed include contamination of the aqueous mucins by the inward movement of lipids,[73] malformation of the glycocalyx,[74-75] and the contamination of the tear film by external debris,[76] in addition to molecular causes such as the influence of Van der Waals attractions.[77]

Norn proposed the measure of corneal wetting as a clinical method to evaluate tear stability, which he defined as the interval between the last blink and the appearance of a break in the tear film when observed under a cobalt blue filter using fluorescein.[78] This measure was later termed tear break-up time (TBUT), and arguably remains the most commonly performed evaluation of the tear film in both clinical and research settings.[79] Despite its popularity, a primary issue with the measurement of TBUT is the use of fluorescein and its possible effects on the outcome of the test. Holly[80] and Norn[81] both acknowledged that instillation of fluorescein does affect the volume of the tear film, and may affect the stability due to contamination of the tear film with fluorescein. Mengher[82] found that the instillation of fluorescein had a significant effect on the TBUT, resulting in a quicker observed break-up; however, a later study by Cho[83] could not replicate Mengher's results and showed no difference in TBUT whether or not fluorescein was instilled. As an alternative to the conventional TBUT, Mengher proposed the measurement of tear break-up using a grid that is projected onto the cornea.[84] This non-invasive measurement could be performed without the use of fluorescein, and was executed by timing the interval between a blink and the first distortions observed in the grid pattern. He named the procedure, quite appropriately, the non-invasive tear film break-up time (NIBUT).[84]

Tear-film interferometry has also been used as an alternative non-invasive technique to measure tear stability.[85-88] King-Smith developed a complex optical system that generates narrowband and broadband images of the pre-corneal tear film via infrared retroillumination of the corneal

surface.[89] The broadband images capture the overlying lipid layer, and when combined with the narrowband images corresponding to the aqueous tear layer, a full-thickness image of the tear film is generated. The observation of local changes in tear film thickness correlate to regional tear break-up, and can therefore be used as a highly sensitive method of measuring tear stability. Interferometry has the added advantage of precisely observing the local and global dynamics of tear break-up.[88]

An indirect non-invasive measure of tear stability is the application of dynamic aberrometry of the ocular surface.[90-92] A smooth, even tear film is known to provide optimal visual clarity,[93] and as the tear film destabilizes and breaks up, a measurable decrease in an individual's function acuity can be observed.[94] Using this knowledge, wavefront analysis has been adapted to measure tear break up by detecting abrupt changes in the higher order aberrations of the ocular surface during the interblink interval. Koh was the first to use a Hartmann-Shack aberrometer to measure tear break up in 20 normals and found almost a 1.5 times increase in higher order aberrations after tear film break-up,[21, 91] a result that was later replicated in a dry eye group.[92] The primary disadvantage of the use of Hartmann-Shack aberrometry to measure tear stability is that the process is pupil-limited, meaning aberrations that occur outside the pupil cannot be measured.

A cut-off value for tear break-up time in the discrimination between normals and those with dry eye was recommended as <10 seconds soon after Norn's first work,[95] and Cho[96] notes that despite a lack of evidence supporting its use, this value has remained entrenched in the literature. Vitali, using fluorescein TBUT, found a sensitivity of 72% and specificity of 62% when <10 seconds was used as a cut-off.[97] When NIBUT was used with the same cut-off, sensitivity and specificity was shown to be slightly higher at 83% and 85% respectively.[84] However, the positive predictive value in both studies was less than 50%,[84, 97] perhaps not surprising knowing the large variability in individual TBUT results[96] and the established poor repeatability of the test.[98-99] Despite the pitfalls, evaluation of TBUT remains the most clinically feasible estimate of tear stability.

1.6 Tear osmolarity

Osmometry is essentially the measure of the concentration of solutes within a solvent, and takes into account the disassociation of solutes in solution, irrespective of their size, density, molecular weight or electric charge.[87] In the case of the tear film, the osmolarity is primarily determined by the content of various cations (including sodium, potassium, calcium and magnesium) and anions (chlorides, phosphates and bicarbonates), the predominant component being sodium chloride.[88] Tear film proteins also contribute to the osmolarity, although their low concentration means their contribution is negligible.[89] The terms osmolarity and osmolality are often used interchangeably in the literature, despite having very different meanings. Osmolarity is an expression of tonicity according to the number of moles per litre of solution (Osm/L), while osmolality describes the number of moles per kilogram (Osm/kg).[90] The low protein content of tears means that molarity is about 5% lower than molality,[88] but for clinical purposes, they are considered equivalent.

A hyperosmolar tear film is thought to be responsible for the symptoms, inflammation and ocular surface damage associated with dry eye.[23, 91-94] Its association as an end-result of both ADDE and EDE make it an ideal candidate as a diagnostic and screening test for the presence of dry eye. The recognized importance of tear osmolarity has necessitated the modification and development of sensitive instrumentation capable of measuring tear film tonicity, including the Clifton™ and Advanced Instruments 3100™ (freezing-point depression osmometers), the Wescor™(vapour-pressure osmometer) and the TearLab™ (an electrical conductance osmometer).

1.6.1 Freezing-point depression osmometers

The early work on the characterization of tear osmolarity in health and disease by Farris, Gilbard, and Hill used the Clifton™ osmometer, an instrument which uses the freezing-point depression technique to determine osmolarity.[100, 103-104, 108-110] Freezing-point depression is based on

the knowledge that the addition of a solute to a solvent will reduce its chemical potential, resulting in a lower freezing point. It is described by the relationship:

$$Osmolarity = \frac{\Delta T}{-1.86}$$

where ΔT represents the magnitude of the temperature depression below 0°C, and -1.86 is the cryoscopic constant for water.[111] The cryoscopic constant is derived from the knowledge that one mole of solute will depress the freezing point of one litre of water by 1.86°C.[111] This highlights how sensitive a freezing-point depression osmometer must be to variations in temperature, as an osmolarity of 300mOsm/L depresses the freezing point a mere 0.00558°C. The Clifton osmometer is capable of measuring osmolarity on volumes as small as 100nL, and consists of a sample holder embedded into a cooling stage and a controller box for the adjustment of the temperature of the unit. A microscope is also used to view the samples throughout the freezing process. Using the Clifton™, osmolarity values for normals have ranged between 302 mOsm/L[100] and 318 mOsm/L,[109] and for those with dry eye, values between 324 mOsm/L[112] to 365 mOsm/L[104] have been obtained.

Although accepted as a gold-standard instrument, the Clifton™ osmometer is a large, complex instrument with the requirement of a specially-trained technician for its operation, and because it is no longer manufactured, its use for the measurement of tear osmolarity is becoming increasingly rare. The Advanced Instruments 3100™ osmometer, which also uses freezing-point depression, has been advocated as a replacement due its smaller size, an automated user-interface, and the requirement of only 500nL of tears for analysis. As it is a relatively new instrument it has not been used extensively, meaning little data is available on its performance. Dalton compared osmolarity values between those with mild-moderate dry eye and age-matched controls, and found a mean osmolarity of 312 mOsm/L in the dry eye group and 305 mOsm/L in the control group, a difference that was not statistically significant.[113]

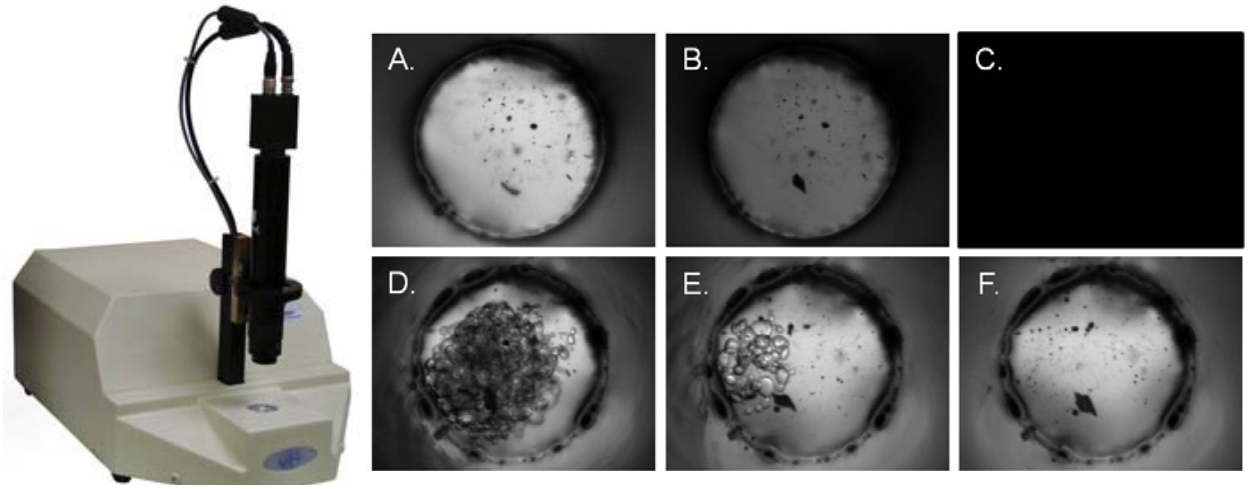

Figure 1-2. Image of Advanced Instruments 3100™ osmometer on left. The image at right displays various stages of the freezing (A – C) and thawing (D – E) process used to calculate sample osmolarity.

1.6.2 Vapour-pressure osmometers

Above the surface of a solution, molecules of solvent are present in the gaseous phase. Sealing the solution in a vessel, such as the measurement chamber in a vapour pressure osmometer, will bring the liquid and vapour phases of the solvent into equilibrium and bring the solvent vapour pressure to a stable value. Under these conditions, the chemical potential of the solution's solvent can be determined by comparing its vapour pressure to that of a pure solvent contained within a reference chamber.[111] Addition of solute to a solvent reduces the solvent's vapour pressure and increases the osmotic pressure, and hence osmolarity, of the solution formed by this addition. The Wescor™ osmometer does not actually measure vapour pressure. Rather, it determines the solvent activity of a solution, which is directly proportional to the vapour pressure.[114] To initiate a measurement, two solutions are introduced to two separate chambers – one being the sample, the other being a reference sample, which is usually pure water. The instrument determines the dew point of each solution then calculates the osmolarity of the sample based on the difference between dew point temperatures. An important limitation of the Wescor™ osmometer is it requires a minimum sample volume of 1000nL

(1µL) to obtain a reading, which is a considerable volume to collect in a subject with ADDE or EDE. Some work has been done to demonstrate that volumes as small as 500nL can be used, but this has only been shown in an in vitro experiment using simple control solutions.[115] For this reason, it is rarely used as a tear osmometer. Karkkainen et al. used the Wescor™ in 100 habitual contact lens wearers with no symptoms of dry eye and found a mean osmolarity of 297 mOsm/kg.[116]

Figure 1-3. Image of the Wescor™ vapour-pressure osmometer.

All freezing-point depression and vapour-pressure tear osmometers suffer from limitations in their collection techniques, as both require a fairly invasive method of collecting tears that is apt to induce a reflex response, artificially lowering tear osmolarity values. Gilbard[100] suggested the use of a glass micropipette with the aid of a biomicroscope, a tedious and time-consuming way of performing a collection, but still the predominant collection technique even today.[108, 113, 116-121] This method runs the risk of inducing a reflex response not just from the repeated contact of the micropipette to the ocular surface, but also in response to the light of the biomicroscope. A second source of error with these methods is the need for sample transfer and storage, as measurement times with the instruments ranges between 80 seconds (in the case of the Wescor™) to 11 minutes (in the case of the Advanced Instruments 3100™ osmometer). This leaves samples susceptible to evaporation, which may inflate osmolarity values. Nelson and Wright evaluated both sources of error, and found that reflex tearing

and storage did have an effect on osmolarity values.[122] To mitigate these confounding variables, a method for the indirect measurement of tear osmolarity using electrical conductance has been proposed.[123]

1.6.3 Electrical conductance

Ogasawara et al. first described the use of a flexible conductimetric sensor that could be placed directly on the eye to measure the conductivity of the tear film.[123] The micro-sensor was minimally-invasive, therefore avoiding a reflex tearing response, and the measurement could be performed in real-time, meaning a tear sample did not have to be collected from the eye for analysis. The conductivity of a fluid is a function of the concentration of ions present, and therefore a reasonable estimate of osmolarity. As the ion concentration increases, the resistance to the flow of electrons decreases, which can be interpreted as an increase in osmolarity. In that same study, osmolarity was measured as 296 mOsm/L in normals, and 325 mOsm/L in those with dry eye, demonstrating the potential validity of the technique for measuring tear osmolarity.[123]

Capitalizing on this concept, the TearLab™ is a handheld nano-osmometer capable of measuring osmolarity on 50 nL sample volumes by measuring electrical conductance. The system consists of a docking station and a pen, to which a chip card is attached. The tip of the chip card is placed into the tear meniscus, and a small channel within the chip card uses capillary action to draw up the tear sample. When the sample is collected, the pen is returned to the docking station for analysis. Analysis is performed by passing an electric current through the tear sample, and the resistance to the flow of current is measured and converted to an osmolarity value. The collection time is approximately 5 seconds, and analysis time is less than 20 seconds, making it an incredibly efficient process relative to previous osmometers. Tomlinson has done work comparing the TearLab™ to the Clifton™, and found osmolarity in controls was 308 ± 6.2mOsm/L (TearLab™) and

310 ± 7.2mOsm/l (Clifton™) and for those with dry eye 321 ± 16.5 mOsm/L and 323 ± 14.7 mOsm/L, results that showed good agreement using Bland-Altman analysis.[120]

Figure 1-4. TearLab™ unit and collection technique with the handheld pen and chip card.

Gilbard and Farris' were the first to suggest a cut-off between normal osmolarity and hyperosmolarity, which they believed to be 312 mOsm/L based on the findings of their first collaboration.[100] Lucca et al., also using 312 mOsm/L as a cut-off, found a sensitivity and specificity of 90% and 95% respectively for the discrimination between those with dry eye and normals.[124] However, later work by Nelson found that using 312 mOsm/L as a cut-off had only 44% sensitivity and 75% specificity,[125] perhaps not surprising because the earlier work used osmolarity as entrance criteria, biasing their cut-off.[100, 124] Since that time, a range of cut-offs have been suggested, with differences attributable to variations in population, entrance criterion and instrument characteristics. The Pisa Criteria for dry eye determined that mild hyperosmolarity was 320 mOsm/L, moderate was 330 mOsm/L and severe was anything greater than 340 mOsm/L.[126] Craig recommended a cut-off of 320 mOsm/L,[127] and Sullivan[128] and Mathers[129] both suggested that 318 mOsm/L would be a reasonable value. As Tomlinson noted, the difficulty with selecting an appropriate cut-off lies in the fact that there is often a significant degree of overlap between osmolarity values in normals and those

with dry eye, particularly in the 300 – 320 mOsm/L range.[130] Taking a different approach, Tomlinson and Khanal performed a meta-analysis of the literature with the hope of determining a referent that would maximize sensitivity and specificity.[130] They arrived at a cut-off value of 315.6 mOsm/L, and when applying this cut-off to an independent sample, found it to have a sensitivity and specificity of 59% and 94%, respectively.[130] The positive predictive value was also high, leading them to suggest a cut-off of 316 mOsm/L, a referent that remains popular.[131]

1.7 Tear meniscus height

The volume of tears present on the ocular surface has been estimated to be 6.2 μL[132] and is regulated by three factors: production of aqueous by the lacrimal gland, drainage via the nasolacrimal duct, and evaporation from the ocular surface. An impairment of aqueous production and/or increase in evaporation may reduce tear volume, ultimately increasing the risk for development of dry eye. For this reason, measurement of tear volume has been indicated as a clinically useful measure to assist in the diagnosis of dry eye.[131] Perhaps the most commonly used test is the Schirmer I test, due to it being a relatively easy and accessible estimation of tear volume. The test is performed by placing a strip of filter paper into the lower conjunctival sac of each eye, and measuring the length of the strip that wets after 5 minutes. A positive test result is when the wetting length is <5mm, although cut-off values between 3mm[117] and 10mm[97] have been cited. Despite its common use, one survey revealed that 74% of ophthalmologists did not believe that the Schirmer I should be a standard of care.[133] This clinical scepticism is related to the demonstrated poor reliability of the test,[134-135] in addition to the myriad of extrinsic factors that can influence results, including the use of anaesthetic,[136] an open versus closed eye,[137] and evaporation.[138] An alternative to the Schirmer test is the phenol red thread test (PRT), which uses a small cotton-thread impregnated with a red dye that is placed into the lower eyelid marginal zone for 15 seconds. This test is preferred because it induces less of a reflex response and

better predicts the basal tear volume. Nonetheless, PRT also suffers from the poor repeatability[99, 135] and sensitivity[139] observed with the Schirmer I.

The tear meniscus has been estimated to contain 75-90% of the volume of tears on the ocular surface,[140] and therefore measurement of meniscus parameters, particularly meniscus height, is predicted be a sensitive indicator of tear volume. The tear meniscus height (TMH) is defined as the vertical difference between where the tear film meets the globe and the upper or lower lid. Most often, the lower tear meniscus is imaged, as it is easiest to view and is not obscured by the lashes, as is the case with the upper tear meniscus. Eugene Wolff, presumably using a slit-lamp to view the meniscus en face (although his method was not adequately reported), was one of the first to publish on the lower tear meniscus height, stating that the average was ≥1 mm.[141] As time has advanced, so have the techniques applied to TMH evaluation, and some of the more frequently reported include a slit-lamp/graticule combination,[142-146] optical pachymetry,[147-148] video capture[147, 149-153] and optical coherence tomography(OCT).[147, 154-162]

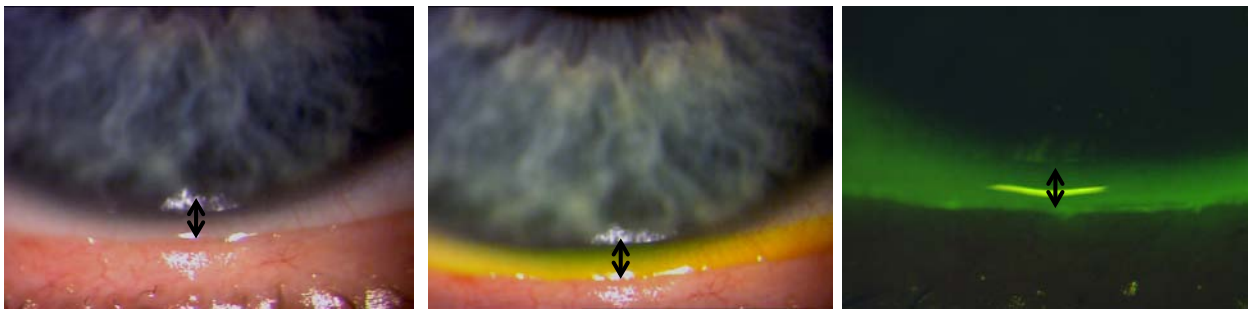

Figure 1-5. Image of the tear meniscus as taken with a slit-lamp and (A) white light (B) white light with sodium fluorescein instilled in the tears and (C) cobalt blue light in combination with a Wratten filter and sodium fluorescein instilled in the tears. The tear meniscus height is indicated by the black arrow.

1.7.1 Slit-lamp and graticule/video capture

The slit-lamp and graticule method is the most feasible of all the techniques for use in clinical practice due to ease of use and accessibility of the required instrumentation. A variation of this method is the use of video capture, whereby images of the TMH obtained from the slit lamp are recorded. These recorded images can either be displayed on a screen and manually measured[149-151] or still images can be outputted for analysis using a variety of software.[147, 152-153] There are a number of procedural differences regarding how these methods should be performed, including whether or not fluorescein should be used to aid visualization, whether the TMH is viewed as a cross-section or en face, and even how best to define the borders of the meniscus. The use of fluorescein is controversial because instillation of fluorescein will increase the tear volume, and thereby artificially inflate the TMH. In addition, there has been some suggestion that fluorescein destabilizes the tear film,[82] which may influence TMH results. Johnson and Murphy explored the effects of fluorescein on TMH and found that 3 minutes after instillation of 5 μL of 2% fluorescein solution, TMH values had returned to baseline, a trend that was maintained to the 5 minute mark.[147] They concluded that any TMH measurements that use fluorescein should be delayed 5 minutes to minimize any effect, although Garcia-Resua found that waiting 5 minutes allowed too much fluorescein to drain away from the ocular surface.[163] Therefore, a more appropriate waiting time after instillation may be 3 minutes to maximize fluorescence, while negating effects on the TMH. The meniscus has been reported to be viewed from the front[143, 145] or as a cross-section[146] and there is some evidence to show that cross-sectional imaging does result in a larger measurement.[147, 152-153] This has been hypothesized to be a result of cross-sectional imaging enabling better visualization of the tails of the meniscus.[147] The top of the meniscus has also been defined as the point at which it is maximally reflective,[148, 163] known as the TMH-R, but this will yield a smaller result than the more commonly accepted definition of the meniscus outlined earlier. Because of the many variations in this technique, there is a range of values

that have been reported for slit-lamp/graticule-measured TMH in normals. In a brief summary of the available literature, Doughty noted a range from 0.15 – 0.46mm, and an average TMH of 0.245mm from 13 reports.[151]

1.7.2 Optical pachymetry

When using the slit-lamp graticule method, minute eye movements, in addition to only being able to measure in 0.1mm steps, may limit the accuracy of measurements. With this in mind, Port modified a Haag-Streit optical corneal pachometer to measure TMH.[148] The pachometer required a custom mounting bracket to position it 90° to where it would normally be positioned to measure corneal thickness, allowing a vertical doubling of the image to be achieved. A disadvantage of this method is that it is difficult to visualize the fine tails of the meniscus frontally, and therefore the authors decided to measure the TMH-R. They found a mean TMH of 0.18mm for measures taken en face at the central lower lid in 66 control eyes, a value that is a slight underestimation due to the use of the TMH-R. Using the same technique, Johnson and Murphy measured TMH in cross-section with and without fluorescein, and en face with fluorescein.[147] Their mean TMH for en face viewing was 0.31mm, much higher than Port's, and was due to the added fluorescein allowing better visualization of the complete meniscus. Cross-sectional viewing yielded a slightly higher TMH at 0.38mm, and the authors noted that viewing the meniscus was easiest using this method. Nonetheless, they advocated the use of en face over cross-sectional viewing due to increased repeatability.[147] Although optical pachometry has demonstrable value for TMH measures, its use will likely be confined to research, as the requirement for a custom-mounting makes its implementation difficult.

1.7.3 Optical coherence tomography

A review of the recent literature makes it apparent that OCT is becoming the preferred method for the measurement of TMH (Table 1). OCT is non-invasive, does not require the instillation

of fluorescein, and gives a cross-sectional, high resolution view of the meniscus, making it in many respects superior to the previously outlined techniques. Izatt was the first to publish on the use of OCT to image the anterior segment *in vivo* in 1994, and suggested OCT would be beneficial for evaluation of the cornea and other anterior segment structures.[164] Eight years later, Jones et al. were the first to document the ability of OCT to image the lower tear meniscus.[165]

OCT is based on measuring the reflectance of light from different layers of an object to create cross-sectional images, akin to a B-scan ultrasound. Light in an OCT system, typically from an infrared superluminescent diode, is passed through a beam-splitter and broken into two arms – a sample arm and a reference arm. The light from the sample arm is directed at the object of interest, and any reflected light is coupled back into the sample arm to be recombined with the reference arm. The combination of reflected light from the sample arm and reference light from the reference arm results in an interference pattern. By scanning a mirror in the reference arm, a reflectivity profile of the sample can be obtained, as is the case with time-domain OCT. It is the reflectivity profile, more commonly known as an A-scan, which contains information about the spatial dimensions and location of structures within the object of interest. By combining multiple A-scans, a two-dimensional representation of the object of interest can be created.

There are two types of OCT that are currently commercially-available: time-domain (TD-OCT) and Fourier-domain (FD-OCT). As noted above, TD-OCT relies on the movement of a mechanical mirror in its reference arm to produce a reflectivity profile. With FD-OCT, the reference mirror is stationary, and instead a spectral pattern is derived from the interference generated between the reference and sample arm. This spectral pattern undergoes Fourier transformation to produce the A-scan. The advantage of FD-OCT over TD-OCT is the use of a stationary reference mirror, which removes mechanical limitations and allows faster scanning times. For example, the fastest TD-OCT

can obtain 2000 A-scans per second, while the fastest FD-OCT is capable of generating 26000 A-scans per second.

There are two characteristics of OCT that make it well-suited for TMH measurement. As mentioned earlier, the wavelength of light used by OCT is in the infrared spectrum, and is usually 800-900nm for retinal OCTs and 1300nm for anterior-segment OCTs (AS-OCT). This is particularly advantageous for the measurement of TMH, as infrared light will not dazzle subjects like white light from a slit lamp will. This eliminates a possible stimulus of reflex tearing, thereby avoiding any influence on the TMH. A second advantage of OCT is the resolution, reported to be between 2 and 20µm. Increased resolution presumably allows for greater accuracy in defining the boundaries of the meniscus, and also provides a larger range of measurement values (i.e. OCT can measure in 0.01 – 0.001mm steps versus a graticule, which is limited to 0.1mm steps).

Retinal-OCTs were the first machines used to measure TMH, as the first AS-OCTs weren't readily available until 2005. Johnson[147] and Srinivasan,[158] both using a first-generation OCT, observed a mean TMH of 0.27mm and 0.162mm respectively in normals. A third-generation OCT (Stratus) used to measure TMH on 20 normals yielded a mean of 0.25mm,[162] in close agreement with the work of Johnson. Because these early OCTs were intended for retinal use, they did not have integrated software capable of analysis of the images. As such, images were exported to third-party software such as PowerPoint,[147] Adobe Photoshop,[162] or custom-software[158] for measurement, which may explain some of the differences observed between studies. Johnson also noted that analysis of images from these early instruments was difficult due to confusion discerning where the eyelid/cornea and tears came together, which he attributed to poor resolution resulting in pixelated images.[147] This led to arbitrary locations being picked for the tails of the meniscus and lowered test repeatability. However, other work has shown that training sessions on defining where the meniscus boundaries are located can eliminate variability in image analysis, and improve repeatability.[166]

With the growing interest in the use of OCT for the evaluation of the anterior segment,[167] a number of papers have evaluated the ability of AS-OCT, using either the Visante, the RTVue-100, or a custom-OCT, to measure the TMH.[71, 154-157, 159-161, 168-169] A comparison study between the Visante and Stratus revealed significant differences between the two instruments, with the Visante biased by an average of 0.05mm greater than the Stratus. Mean TMH values in control groups reported in other studies, 0.331mm[71] and 0.40mm,[155] are also higher than those reported using retinal-OCTs.[147, 158, 162, 166] Similar trends in TMH values are observed using the RTVue-100 FD-OCT, a retinal-OCT with interchangeable lenses that render it capable of capturing images of the anterior segment. Kim et al. used the RTVue-100 to image both lower menisci in those who had undergone anophthalmic surgery in one eye, and found that the meniscus along the prosthetic eye was significantly lower than the non-operated eye (0.200mm and 0.261mm, respectively).[168] Zhou used the same instrument to evaluate TMH in 20 normal subjects and found a mean of 0.285mm.[161] Wang has developed a custom AS-OCT that is capable of imaging both the upper and lower meniscus simultaneously in real-time.[169] It has been used in a variety of studies that have used TMH measures to understand tear dynamics, and TMH values in control groups have ranged between 0.240mm and 0.339mm.[154, 157, 159-160] The trend towards increased measures of TMH using AS-OCT versus retinal-OCTs may be secondary to dewarping algorithms that AS-OCTs employ as a means of converting the image from optical space to physical space. A dewarping calculation consists of two factors: a shape factor and distance factor.[170] When the scan beam length of the AS-OCT is greater than 3mm, it is unlikely that all incident beams from the OCT will be perpendicular to the corneal surface. This results in bending, or warping, of light which must be compensated for using the shape factor. The distance factor relates to the refractive indices of the target tissue. To make correct physical distance measurements, dimensions measured must be divided by the refractive index of the media.

Table 1 briefly summarizes papers published between 2007 and 2010 that have evaluated TMH measures in either controls and/or those with dry eye. The mean TMH in normals for the 15 papers is 0.266mm, and falls in between the mean reported in Johnson and Murphy's review of TMH literature in 2005 (0.28mm),[147] and that reported by Doughty in 2002 (0.245mm).[151] It is interesting to note that if one only considers those published results that have used AS-OCTs, the average TMH jumps to 0.295mm. The gradual increase in TMH values may be related to the concurrent improvement in image quality and resolution that newer instruments are capable of. The current generation of OCTs, particularly FD-OCTs, have a resolution that is considerably higher than other imaging techniques, making it easier to define the meniscus borders. Meniscus parameters that are easier to identify should also result in improved repeatability, and studies have shown that the repeatability of an FD-OCT is superior to other instruments.[161, 168]

Author	Year	Method	Control (n)	Control, mm (mean ± SD)	Dry eye (n)	Dry eye, mm (mean ± SD)
Ibrahim et al.[155]	2010	OCT (Visante)	27	0.40 ± 0.17	24	0.25 ± 0.08
Yuan et al.[160]	2010	OCT (Custom)	30	0.268 ± 0.097	25	0.227 ± 0.093
Chen et al.[154]	2010	OCT (Custom)	20	0.251 ± 0.036	20	0.192 ± 0.029
Shen et al.[157]	2010	OCT (Custom)	35	0.240 ± 0.053		
Koh et al.[71]	2010	OCT (Visante)	11	0.331 ± 0.085		
Kim et al.[168]	2010	OCT (RTVue-100)	31	0.261 ± 0.087		
Garcia-Resua et al.[163]	2009	Slit-lamp w/ graticule	34	0.250 ± 0.08		
Zhou et al.[161]	2009	OCT (RTVue-100)	20	0.285 ± 0.08		
Savini et al.[156]	2008	OCT (Visante)	26	0.28 ± 0.12		
		OCT (Stratus)		0.23 ± 0.07		
Bitton et al.[171]	2008	OCT (OCT2)	15	0.117 ± 0.03	15	0.143 ± 0.05
Harrison et al.[172]	2008	Slit-lamp w/ mm ruler	15	0.40 ± 0.09	15	0.30 ± 0.07
Wang et al.[159]	2008	OCT (Custom)	36	0.339 ± 0.149		
Srinivasan et al.[158]	2007	OCT (OCT2)	20	0.142 ± 0.016	20	0.133 ± 0.012
Uchida et al.[173]	2007	TearScope	17	0.22 ± 0.065	27[a]	0.13 ± 0.042
Kawai et al.[174]	2007	Slit-lamp w/ fluorescein	19	0.24 ± 0.08	14	0.17 ± 0.07
			MEAN	0.266mm		0.193mm

[a] = dry eye group consisted solely of those with Sjögren's syndrome

Table 1-1. Tear meniscus height values reported in the literature between 2007 and 2010 for normals and those with dry eye.

Various studies have commented on the utility and cut-off values for TMH measures in the diagnosis of dry eye. Lamberts suggested that a cut-off of <0.1mm was suitable for the diagnosis of dry eye based on the finding that over 93% of TMH values were greater than 0.1mm in 86 normals. On the opposite extreme, Mainstone used a video-capture to determine that a cut-off of ≤0.35mm had

93.3% sensitivity and 66.7% specificity. The range of cut-off values is reflective of the variation observed in TMH measures between studies, making it difficult to select a value with appropriate predictive value. The mean TMH in the dry eye group from Table 1 is 0.193mm, which might suggest that a cut-off of approximately 0.2mm might be appropriate. However, with the apparent increase in measured TMH that is observed with the newer, and more frequently used AS-OCTs, 0.2mm may result in under-diagnosis of dry eye. One advantage of the increasing use of only two instruments for TMH measurement is that there will be more consistency in the analysis techniques employed by different studies, as both the RTVue-100 and Visante have standard integrated analysis software. This may lead to a more universally accepted cut-off being derived in the future. Regardless, TMH demonstrates acceptable sensitivity and specificity[155, 157, 175] to be a valuable test in the diagnosis of dry eye.

1.8 Conclusion

As the DEWS definition of dry eye states, dry eye is a complex, multi-factorial disease that involves dysfunction of the tear film on multiple levels.[24] This complexity makes it incredibly difficult to correctly diagnose dry eye, as there are multiple aspects of the lacrimal functional unit to consider. The ideal test for the screening and diagnosis of dry eye would have high sensitivity and specificity, in addition to a high positive predictive value. More importantly, it would be easy to perform in a clinical setting, with minimal instrumentation and maximum efficiency. As practitioners, we often rely heavily on a patient's subjective complaints to make the diagnosis. However, objective tests add information regarding severity and possible aetiology, which factor significantly when trying to plan treatment or chart the progress of the disease.

The TMH has been indicated as a clinically useful estimate of the volume of the tear film.[25] If done without the use of fluorescein, it is non-invasive, and it has been shown to have relatively high sensitivity and specificity for the diagnosis of dry eye.[155, 157, 175] For the aforementioned reasons, OCT

is ideally suited to measure TMH, and its high resolution and integrated software presumably make the measurement process easier and more accurate. Although TMH is a valuable objective test that can aid in the diagnosis of dry eye , changes in our understanding of the condition is leading to other tests that may have greater sensitivity and specificity. It has been suggested the majority of symptoms and signs of dry eye are primarily due to hyperosmolarity of the tear film.[104, 118] Because of this, osmolarity has been postulated to be the new "gold standard" for the diagnosis of dry eye, and Khanal showed that tear osmolarity performed well as a single diagnostic test for the diagnosis of dry eye.[176] However, a number of factors have prevented osmolarity from becoming a standard clinical test in the diagnosis of dry eye, namely the need for large sample volumes that are invasively collected, bulky and complex instrumentation, and inefficient analysis methods. The TearLab is poised to become the first instrument capable of measuring tear osmolarity in a non-invasive, time efficient manner, but little is known about its performance and capabilities.

The following chapters present data on measures of tear film parameters using two new technologies, the RTVue-100 FD-OCT and the TearLab nano-osmometer. Of particular interest is each instrument's performance in both the normal and dry eye population.

Tear Meniscus Height Determination Using the OCT2 and the RTVue-100

This chapter is published as follows:

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	Concept / Design	Recruitment	Acquisition of data	Analysis	Write-up / publication
Keech	Y	Y	Y	Y	Y
Flanagan	Y	-	-	-	-
Simpson	Y	-	-	Y	-
Jones	Y	-	-	-	-

Chapter 2

Tear Meniscus Height Determination Using the OCT2 and the RTVue-100

2.1 Introduction

It is well-established that the ocular surface requires a certain volume of tears to maintain a trophic environment. The tear film bathes the ocular surface, freeing it from debris, supplying valuable nutrients and maintaining a stable refractive interface.[1, 2] Deviations in the volume can lead to symptoms of ocular discomfort, including burning, foreign body sensation, ocular fatigue and visual blurring.[3] Despite the importance of tear volume, there is a distinct lack of test availability that can accurately quantify tear volume. Currently, one of the most commonly used methods is the Schirmer I test, although it has been shown to have poor diagnostic sensitivity[4] and repeatability.[5] Another disadvantage of the Schirmer I test is its invasive nature, which has the potential to compromise any results via the induction of reflex tearing.

With this in mind, the tear meniscus height (TMH) has been indicated as a clinically useful estimate of the volume of the tear film.[6] If done without the use of fluorescein, it is non-invasive, and its has been shown to have relatively high sensitivity and specificity.[7] Methods of measuring TMH have included the graticule method,[8-10] image capture,[11-14] optical pachymetry,[10, 15] and optical coherence tomography (OCT).[7, 16-20] The use of OCT is becoming increasingly widespread because of the high resolution images that it produces, which makes analysis easier and, presumably, more accurate. The majority of published results on measuring the TMH using OCT have used conventional time-domain OCT (TOCT).[16-20] Recently, Optovue Inc. released a commercially available spectral-, or Fourier-, domain OCT (SOCT) capable of imaging the anterior segment. SOCT provides a higher resolution image that can be captured in a short time frame compared to TOCT. It

uses two lens attachments, the Cornea/Anterior Module-Short (CAM-S) and CAM-Long (CAM-L) to obtain anterior segment images. The CAM-S attachment provides a high magnification view, while the CAM-L provides a wider viewing angle but a slightly decreased resolution (Figure 2 – 1). In addition, integrated analysis software is provided, which obviates the need for the data to be exported to third-party software for subsequent analysis.[16, 18, 20, 21]

The purpose of this study was to compare TMH measurements obtained using TOCT (OCT2, Carl Zeiss Meditec, Dublin, CA, USA) to SOCT (RTVue-100, Optovue, Freemont, CA, USA).

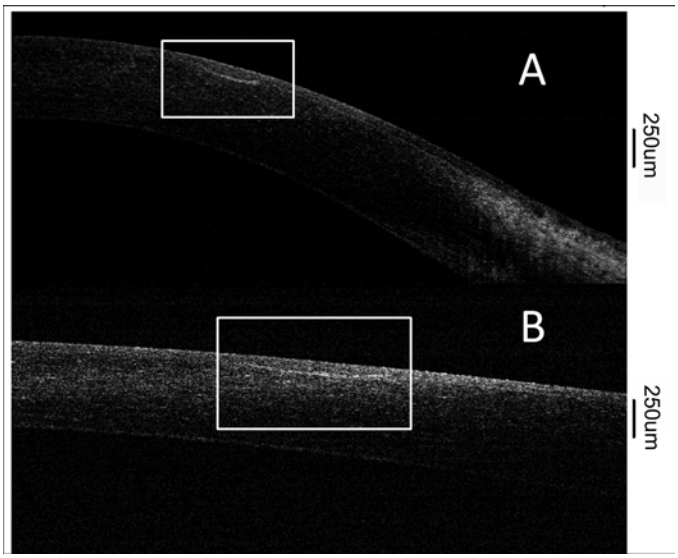


Figure 2-1. Native image of corneal scar taken with the RTVue-100 CAM-L lens (A) and CAM-S lens (B). The CAM-S lens provides higher magnification, while the CAM-L lens produces an image with a wider field of view but reduced magnification.

2.2 Methods

The protocol used for this study received ethics approval from the Office of Research Ethics at the University of Waterloo. 50 healthy subjects (16 males and 34 females) with a mean age of 33.2 years (range 19-62 years) were recruited to participate in the study. Subjects were excluded from the study if they had a history of refractive surgery, exhibited the presence of conjunctivochalasis, had

worn contact lenses in the past 12 hours, or used artificial tear drops over the previous 6 hours. All subjects gave their informed consent according to the Declaration of Helsinki.

The same examiner (AK) imaged the lower TMH of the right eye in all patients using both the OCT2 and the RTVue-100. Three scans were taken using the OCT2, and six scans were taken using the RTVue-100, three using the CAM-S lens and three using the CAM-L lens. The order in which the scans were carried out was randomized. A small mark was placed below the lash margin of the lower right lid at approximately the 6 o'clock position to ensure that the scans were completed in the same position using all imaging modalities. When applying the mark, care was taken to ensure that no contact was made with the ocular surface, thereby avoiding interference with the normal tear film.

The nine scans were performed consecutively within a ten-minute period in a temperature (25°C ± 1°C) and humidity (13% ± 2%) controlled room. Both instruments were placed in the same room, side-by-side, to expedite the measurement session. The lights were dimmed and equipment moved away from ventilation ducts to avoid the induction of reflex tearing. Patients were asked to look straight ahead and blink as necessary to avoid ocular surface desiccation.

The settings and methods used to image and analyze the TMH with the OCT2 have been described elsewhere.[20] Briefly, a beam of 1.13mm vertical height was focused onto the ocular surface centered within the lower lid marking. We attempted to capture the images within the first second immediately following a blink. The raw image data were exported to proprietary analysis software capable of measuring the TMH, where it was analyzed by a masked examiner. The height of the meniscus was taken as the vertical difference between the point at which the tail of the meniscus intersected with the cornea superiorly and the lower lid inferiorly. The default scan settings were used on the RTVue-100 OCT, meaning a 2.0mm vertical beam was used with the CAM-S lens and a 6.0mm vertical beam was used with the CAM-L. All image analysis was performed using the built-in

calliper tool as part of the RTVue-100 software package using the same criteria as described above to determine the TMH.

After completing the study, both instruments were calibrated against two different slit-lamp graticules, each comprised of an internal ruler divided into 0.1mm intervals. The graticules were mounted onto a lens stand, and imaged using the OCT2, and CAM-S and CAM-L SOCT lenses using the settings outlined above. A 1mm section of each image was randomly chosen and ten consecutive intervals were measured (Figure 2 – 2).

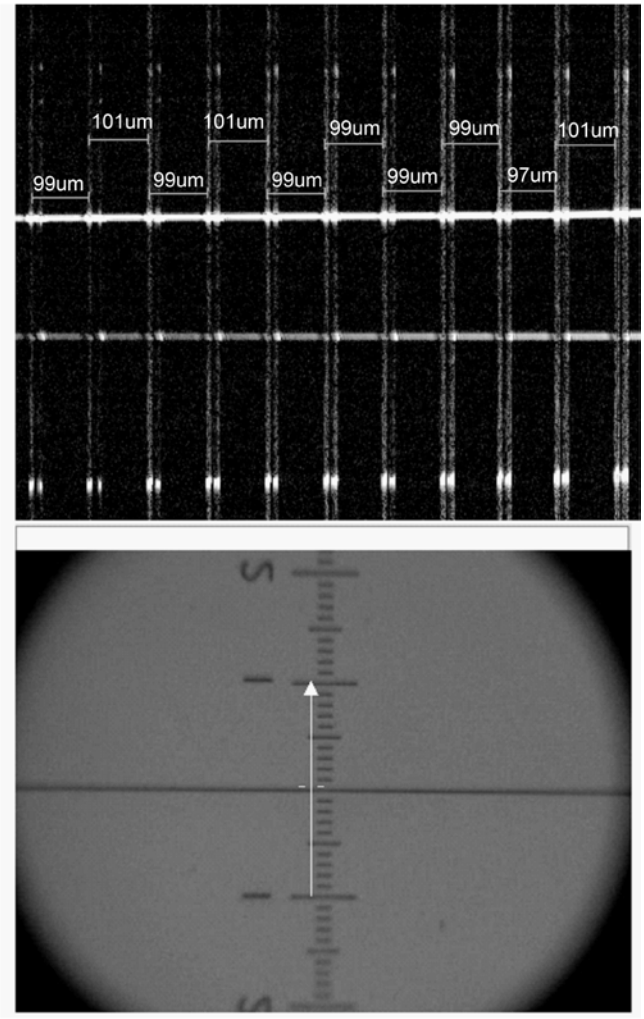


Figure 2-2. A 2mm section of the graticule as imaged by the CAM-S lens. Due to excess noise levels when imaging the graticule surface with either of the SOCT lenses, the focal point was set ahead of the graticule, allowing the interval between reflections of the lines to be measured.

2.3 Statistical analysis

Analysis was performed using Statistica version 7 (StatSoft, Tulsa, OK, USA) and a free online statistical calculator.[22] A repeated measures ANOVA was calculated to evaluate differences between the three instruments and to determine if a difference between measurement sessions existed, followed by analysis using Tukey's Honestly Significant Differences (HSD) test. The relationship between the three measurement methods was analyzed by Lin's concordance of correlation

coefficient[23] and Bland-Altman analysis.[24] A p value of less than 0.05 was considered statistically significant.

2.4 Results

Calibration testing showed that the mean interval measured on the first graticule using OCT2 was 0.099 ± 0.013mm and 0.102 ± 0.006mm on the second graticule. The CAM-S lens measured 0.099 ± 0.003mm/ 0.099 ± 0.001mm and the CAM-L measured 0.100 ± 0.003mm/0.100 ± 0.005mm for the mean interval of the first/second graticule respectively. Calibration also revealed that the scan height setting of 1.13mm produced a beam that was 1.40mm high. Based on this result, all measures taken with the OCT2 were scaled accordingly.

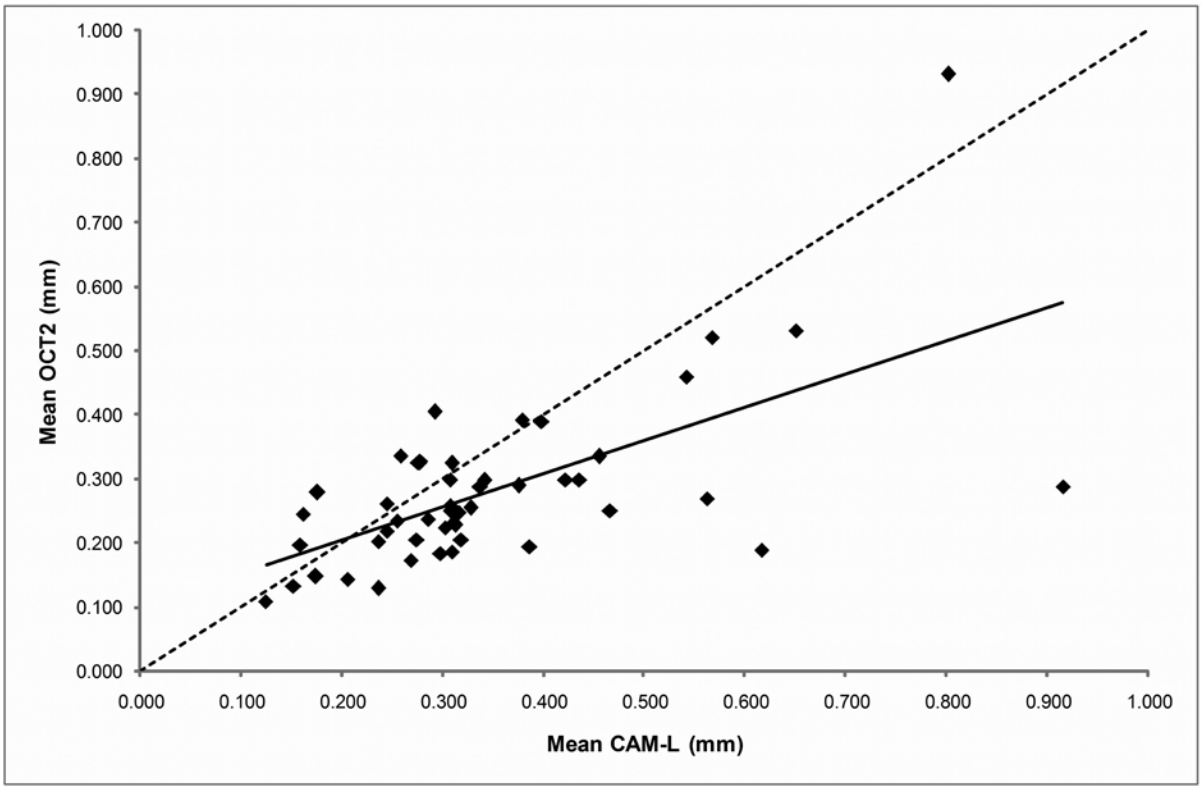


Figure 2-3. Lin's concordance of correlation coefficient comparing the OCT2 and CAM-L lens, ρ_c = 0.5566. Note that there is closer agreement between instruments for TMH values measuring between 100µ and 200µ, and the tendency of the CAM-L lens to give higher measurements as the mean TMH increases.

The mean TMH as measured by the OCT2 was 0.280 ± 0.139mm, while the mean TMH measured using the CAM-S and CAM-L lens was 0.354 ± 0.163mm and 0.345 ± 0.167mm respectively. Bland-Altman analysis showed a low level of agreement with both the CAM-S and CAM-L lens in comparison to the OCT2. The 95% limits of agreement (LOA) ranged between -0.138 and +0.285mm for the CAM-S and -0.185 and +0.315mm for the CAM-L. Lin's concordance of correlation coefficient (ρ_c) was 0.6402 for the CAM-S and 0.5566 for the CAM-L (Figure 2 – 3). When comparing the CAM-S directly to the CAM-L, Lin's CCC showed a high strength of agreement with ρ_c = 0.8944. Repeated measures ANOVA showed a statistically significant difference

between measures taken with the OCT2 and CAM-S ($p<0.001$) and OCT2 and CAM-L ($p<0.001$) (Figure 2 – 4). This result was confirmed using Tukey's HSD, with a $p<0.001$ when comparing the OCT2 to the CAM-S and CAM-L respectively. A difference between the CAM-S and CAM-L lens was not demonstrated ($p=1.0$). There was not a statistically significant difference between the three measurements taken using each instrument ($p=0.101$), although the measured TMH did increase slightly from the first to third measurement.

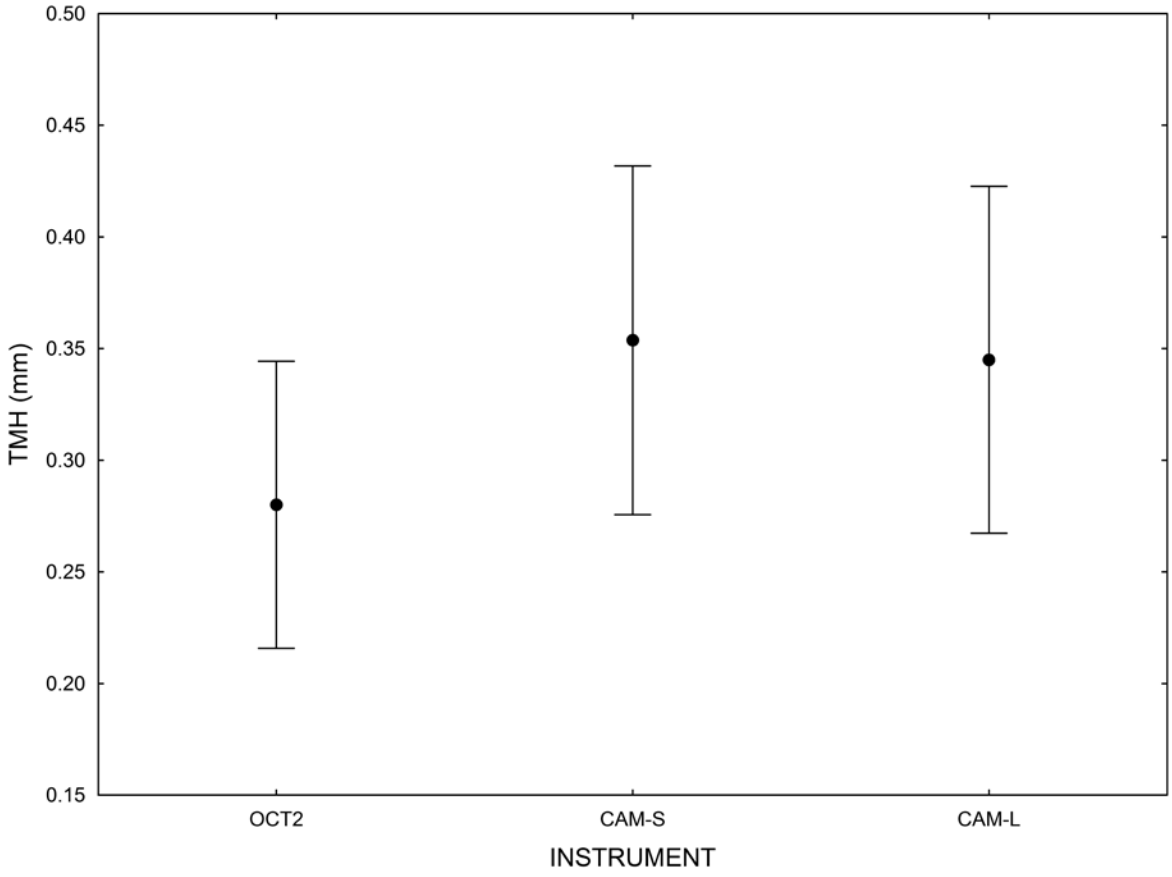


Figure 2-4. Repeated measures ANOVA showing poor agreement between the OCT2 and both the CAM-S and CAM-L (both $p < 0.001$) TMH measures. There was no significant difference between the CAM-L and CAM-S values ($p=1.0$). Vertical bars denote the 95% confidence intervals.

2.5 Discussion

The data analysis showed a significant difference between the measurements obtained with conventional TOCT as compared to SOCT. The OCT2 yielded a mean TMH of 0.280mm, while the mean TMH obtained using the CAM-S and CAM-L lens was 0.354mm and 0.345mm respectively. Although higher than the OCT2, the two SOCT results were concordant, and showed a high strength of agreement based on the results from Lin's concordance correlation coefficient ($\rho_c = 0.8944$). Nonetheless, SOCT TMH measures were almost 20% higher than the results obtained with the OCT2, and do not agree with any published data on TMH obtained with OCT. There is only one other published result that looked at the ability of a commercially-available anterior segment OCT (AS-OCT) to measure TMH.[16] In that study, a mean TMH of 0.28mm was reported. Although our measured TMH was higher, it does point out that anterior segment-specific OCTs have thus far tended to yield a higher measured TMH when compared to traditional OCTs designed for posterior segment analysis.

The difference in measured TMH between posterior segment OCT and AS-OCT has been postulated to be due to AS-OCTs using complex dewarping algorithms when converting an image from optical space into physical space.[16] There are two aspects to the dewarp calculation: a shape factor and distance factor.[25] When the scan beam length is greater than 3mm, it is unlikely that all incident beams from the OCT will be perpendicular to the corneal surface. The result is bending, or warping of light, which must be compensated for using a shape factor. The distance factor relates to the refractive indices of the target tissue. To make correct physical distance measurements, dimensions measured must be divided by the refractive index of the media. We suspect that the RTVue-100 performs one or both of these dewarping calculations, which may alter the processed image and artificially increase the measured TMH. However, without access to commercially-

sensitive information about the design of the software and instrument, we are unable to definitively surmise this.

A second explanation for the higher measured TMH using SOCT may be the increased resolution provided by SOCT. Figure 2 – 5A shows the image as obtained with TOCT. The large pixels make delineation of the borders of the meniscus difficult, and the examiner is often left making assumptions as to where the meniscus meets the cornea and lower lid. This same observation was made in an earlier paper, which showed that significant variation in image analysis can be present due to reduced resolution.[20] Figure 2 – 5B shows an image obtained with the CAM-S lens that has been broken up into two zones, A and B. Zone A correlates with what is likely resolved in a conventional TOCT image and zone B shows the "fine tail" of the upper section of the tear meniscus . It is this zone that is plausibly indefinable in a TOCT image, and we surmise results in the higher measurement values obtained using SOCT. For example, in Figure 2 – 5B, zone B adds an additional 223μ to the measured TMH.

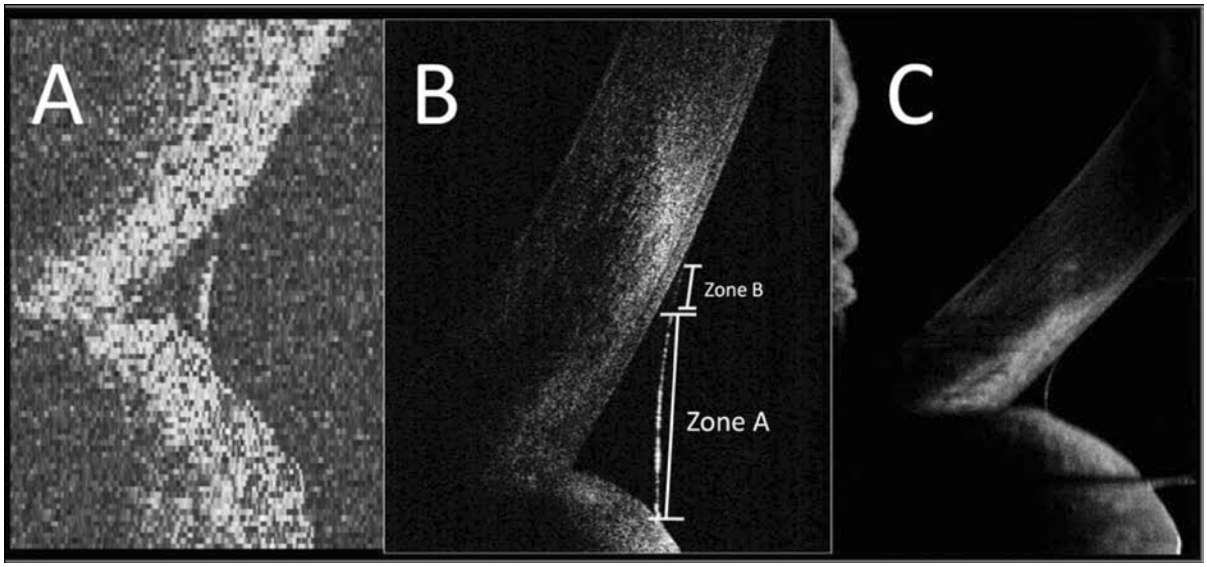


Figure 2-5. TM of the same eye imaged using the OCT2 (A), CAM-S (B), and CAM-L (C). The images have been cropped and re-sized for comparison purposes, and magnification and scale are not equal for all three images. Figure 2-5B shows an image obtained with the CAM-S lens that has been broken up into two zones, A and B. Zone A correlates with what is likely resolved in a conventional TOCT image and zone B shows the fine tail of the tear meniscus . It is this zone that is plausibly indefinable in a TOCT image, and results in the higher measurement values obtained using SOCT. In Figure 2-5B, zone B adds an additional 223µ to the measured TMH.

The high degree of variability between instruments as demonstrated by Bland-Altman analysis can likely be attributed to the dynamic nature of the tear film. Tear flow has been estimated to be 0.30µL/min and has a turnover rate of 16%/min.[26] These factors, coupled with evaporative effects, will cause a degree of variability if measures are not taken at the same time point. We attempted to minimize this by taking the scan immediately after a blink.

This study also highlights the importance of calibrating instruments and software before use. Failure to perform calibration would have resulted in the OCT2 mean TMH measuring 0.226mm, which is 25% lower than the calibrated measurement. Interestingly, it is the uncalibrated TMH that correlates well with other published results using similar instruments that had not been calibrated.[16, 18-

[20] Bitton et al., also using a second generation OCT and the same custom measurement software, obtained a mean TMH of 0.24mm.[20] A more recent study by Savini used the Stratus OCT and found a mean TMH of 0.23mm.[16] Although some may question why an older OCT was chosen in this particular study, the fact that our uncalibrated TMH results found with the OCT2 concur with the Stratus should show the machines to be comparable. This is only the second study that the authors are aware of that have calibrated the OCTs used throughout the study to measure TMH.[21]

SOCT demonstrated a number of advantages over conventional TOCT. Primarily, the increased resolution of the RTVue versus the OCT2 (5µ vs. 10µ) makes image analysis easier, because there is less discrepancy regarding the identification of the borders of the tear film, a problem identified in another study.[20] This improves consistency in image analysis within and between observers. Secondly, the ease and speed with which an image can be captured is a significant benefit, as it is probably more reflective of the natural state of the tear film. The scan time of the RTVue is 0.16 second, considerably shorter than the 1-2 seconds that it takes for the OCT2. This increased time to acquire the scan may also lead to evaporation of the tear film, reducing the volume, and hence TMH measurements, obtained with the OCT2. Another major advantage of the RTVue over OCT2 is the provision of integrated analysis software. This eliminates the need to export the images and does away with the need for development of software capable of analysis. It also allows for comparison and consistency during multi-center studies, as the software is standard on each instrument.

Future research needs to establish the causative factors behind the differences in measured TMH between TOCT and SOCT. In the event that it is demonstrated that SOCT over-estimates TMH, it would be ideal if a correction factor could be derived that will allow for accurate measurements and the use of either instrument interchangeably.

2.6 Acknowledgements

The authors would like to thank Dr. Natalie Hutchings and Marc Schulze for their assistance with the statistical analysis. They would also like to acknowledge Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Foundation for Innovation (CFI) as funding sources for the equipment.

Chapter 3
Impact of time between collection and collection method on human tear fluid osmolarity

3.1 Introduction

In the early 1950's, Balik first proposed, but was unable to demonstrate, a link between tear hyperosmolarity and keratoconjunctivitis sicca (KCS), or dry eye.[1] Despite his failure to do so, the idea that increased osmolarity was a primary causative factor behind the development of dry eye persisted and throughout the 1970's and 1980's, Gilbard,[2-6] Farris,[2, 3, 5, 7-9] and Benjamin[10-12] produced an immense amount of data on osmolarity values in normals and those with KCS. Their work provided the foundation for the Dry Eye Workshop (DEWS) report on the definition and classification of dry eye, which recognized tear hyperosmolarity as a core mechanism.[13] Despite an established role, the measurement of tear osmolarity has not been routinely undertaken in clinical practice due to a number of technical limitations of the available instrumentation.

Much of the initial work by Gilbard and Farris was accomplished using the Clifton nanolitre osmometer [Clifton Technical Physics, Hartford, NY], which uses the freezing-point depression technique to determine osmolarity.[2-4] Although a reliable instrument,[14] it requires a trained technician to process samples and is too large to be used outside the realm of research. Vapor-pressure osmometry has also been used with some success,[15-17] but is hampered by the requirement of large tear volumes to yield an accurate measurement. A major disadvantage of both instruments is the collection technique employed to gather a sufficient tear sample. The collection technique developed by Gilbard,[18] and subsequently used by others,[19, 20] uses a slit-lamp to guide a glass capillary tube into the lower meniscus to collect the tear sample. This method is invasive and apt to induce reflex tearing, secondary to mechanical or visible light stimulation of the eye.

Ogasawara et al. devised a flexible sensor capable of measuring osmolarity by responding to the conductivity of the tear film, and used the technique to show an increased osmolarity in those suffering from KCS versus a control group.[21] The primary advantage of measuring tear film conductivity over other osmometry methods is that the volume required is independent of the osmolarity, meaning a small volume is sufficient. This makes it an ideal methodology for tear collection in those suffering from aqueous-deficient dry eye. Building on Ogasawara's work, in 2007 TearLab released a handheld nano-osmometer capable of measuring tear osmolarity on 0.05 µL sample volumes. The device utilizes a unique chip card system which non-invasively collects and processes tear samples within 60 seconds. This collection technique overcomes two of the limitations of the glass capillary tube method. First, collection of a sufficient tear sample takes less than one second using the TearLab, thereby minimizing the potential influence of reflex tearing. Secondly, the analysis is performed within the chip card and the sample does not need to be transferred for analysis, which mitigates any evaporative effects. Although the TearLab appears to overcome a number of technical and methodological short-comings of other methods outlined above, little is known about its performance in a dry eye or normal population. The present study consisted of two-phases aimed at validating the use of the TearLab nano-osmometer in a clinical setting. Secondary outcome measures included the ideal time interval between consecutive collections that will yield reproducible results, and the effect that the collection method, namely glass capillary tube, has on tear film osmolarity.

3.2 Methods

The study received approval from the University of Waterloo's Office of Research Ethics, and in adherence to the tenets of the Declaration of Helsinki, informed consent was obtained from all subjects before enrolment. Exclusion criteria for the study was the use of artificial tears six hours prior to any study visit, and participants were asked to refrain from wearing contact lenses while enrolled in the study. After subjects were enrolled, they completed the Ocular Surface Disease Index

(OSDI), a standardized questionnaire used to categorize subjects into dry eye or control groups.[22] An OSDI score of ≥20 placed a subject into the dry eye group.

During phase I of the study, 10 subjects were recruited into each of the control and dry eye groups. Subjects attended three visits over a five day period, with the time of each visit kept the same to eliminate any diurnal variations of the tear film. In the course of a given visit, eight tear collections were taken from each eye using the TearLab, the first four separated by a 15 minute interval, and the final four separated by a one minute interval. A 15-minute washout period was given between the fourth and fifth tear collection to allow the tear film to equilibrate. At the conclusion of the visit, a tear break-up time (TBUT) was measured on each eye in triplicate via instillation of sodium fluorescein.

For phase II,15 subjects were enrolled into the control group and 15 into the dry eye group using the same criteria as above. The second phase consisted of four visits attended over a two week period. Eight tear collections were performed on each eye at each visit. The first four collections were performed using the TearLab and were collected a 90-second intervals. This was followed by a 15-minute washout period, whereupon 5µL was collected from both the right and left eye using a glass capillary tube [Drummond Scientific, Broomall, PA, USA] (GCT).

When collecting tears using the TearLab, subjects were instructed to direct their gaze superonasally, and the collection tip of the chip card was gently placed into the tear meniscus at the lateral canthus. The tip was moved in and out of the meniscus to stimulate the capillary action, care being taken to avoid contacting the ocular surface. After the sample was successfully collected, it was immediately returned to the docking station for measurement. The collection process using the GCT was similar to that of the TearLab. Subjects looked superonasal, and the tip of the GCT was placed into the tear meniscus at the lateral canthus. Care was taken to avoid contacting the ocular surface and lids; however, if the investigator (AK) or participant observed any reflex tearing, the collection

process was stopped for 90 seconds to allow for equilibration of the tear film. Collection continued until either 5µL of tear film was obtained or 7 minutes passed, the latter guideline used to minimize evaporative effects. When collection was completed, the sample was immediately transferred to 0.1mL PCR tubes (Axygen Scientific Inc., Union City, CA, USA), spun down for 7 seconds at approximately 6500rpms, and placed on ice. Osmolarity of the tear sample was measured by transferring 500nL onto a clean 1" square of Parafilm (Pechiney Plastic Packaging Company, Chicago, IL, USA) and immediately collecting the dispensed sample with the TearLab. The measurement process was repeated four times on each tear sample.

3.3 Statistical analysis

Data from phase I and phase II was entered into Microsoft Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) and subsequently exported to Statistica version 7 for analysis (Statsoft, Tulsa, OK, USA). The results generated in both phases from the TearLab and through the measurement of TBUT were evaluated using a repeated-measure ANOVA. A t-test was used to compare OSDI scores between control and dry eye groups. Relationships between variables, namely OSDI score, osmolarity and TBUT, were quantified through the use of Pearson's correlation coefficients or non-linear regression analysis, and $r > 0.60$ was considered to be a significant correlation. The coefficient of variation (CV) was determined for both control and dry eye groups in phase I of the experiment at 15 minute and 1 minute intervals. Repeatability of osmolarity results for each time interval was assessed through the calculation of intraclass correlation co-efficients (ICC) for mixed models. A p-value equal to or less than 0.05 was considered to be statistically significant.

3.4 Results

Phase I enrolled four males and sixteen females for participation, with a mean age of 42.8 ± 16.4 years (range 22 – 73). Mean osmolarity of the control group across all measures was 287.9 ± 7.5

mOsm/L and for the dry eye group, mean osmolarity was 297.8 ± 14.7 mOsm/L, which was significantly different (p=0.007) [Table 3 – 1]. OSDI scores and TBUT were also noted to be significantly different between the two groups (p<0.0001 and p=0.029). No significant difference was observed between left and right eye measures (p=0.202), 15 minute versus 1 minute intervals (p=0.169), consecutive visits (p=0.890) and consecutive measures (p=0.158). The effect of time interval on consecutive measures was evaluated for both groups, and no significant difference was noted (p = 0.99), a result supported by Tukey HSD *post hoc* testing (Figure 3 – 1).

	Variable	**Control (mean ± SD)**	**Dry eye (mean ± SD)**	***p***
Phase I	*Osmolarity (mOsm/L)*	287.9 ± 7.5	297.8 ± 14.7	0.007
	OSDI	7.1 ± 4.4	40.0 ± 14.8	<0.0001
	TBUT (sec)	10.76 ± 7.76	5.03 ± 4.42	0.029
Phase II	*Osmolarity (TearLab™)*	287.7 ± 8.01	295.8 ± 12.5	0.0058
	Osmolarity (Glass capillary tube)	282.4 ± 9.8	291.1 ± 9.9	0.041
	OSDI	7.09 ± 4.71	36.53 ± 11.89	< 0.001
	TBUT (sec)	20.82 ± 15.75	5.96 ± 2.68	< 0.001

Table 3-1. Summary of comparisons between control and dry eye group for phase I and II data.

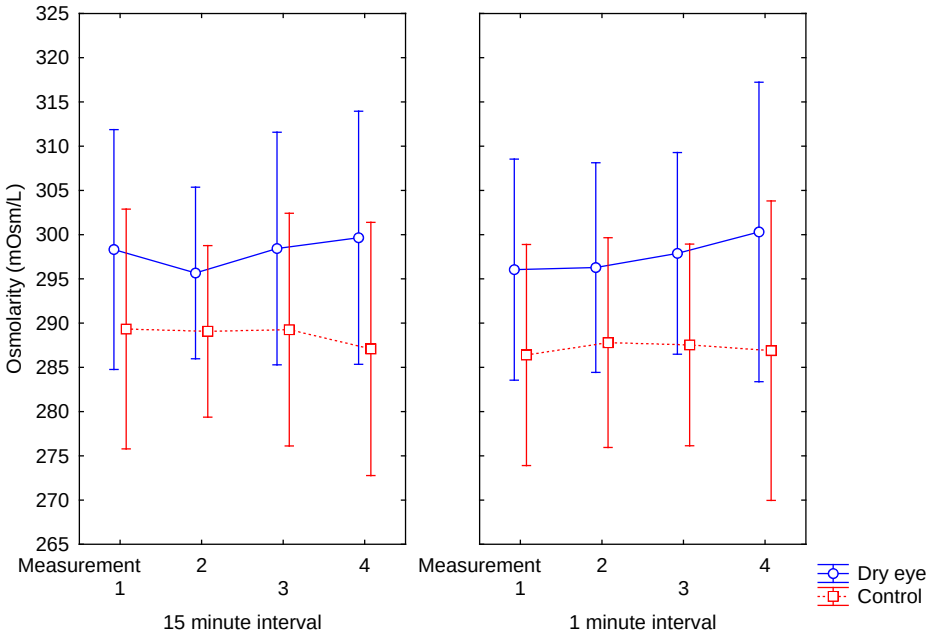


Figure 3-1. Mean osmolarity for each of the four measurements at 15 minute intervals and 1 minute intervals.

Because no difference was observed for osmolarity measures between the right and left eye, to simplify the statistical analysis the following results are described using osmolarity and TBUT measures from the right eye only. The mean coefficient of variation (CV) for osmolarity measures at 15 minute and 1 minute intervals was 2.19/2.09% for controls and 3.48/3.31% for dry eye subjects. Pearson's correlation coefficients were low for comparisons between a subject's OSDI score and either TBUT or osmolarity, but by applying a bilinear fit,[23] we were able to show a strong linear relationship between TBUT and osmolarity values (R = 0.85) [Figure 3 – 2]. ICC values were high for both 15 minute (ICC = 0.899) and 1 minute (ICC = 0.923) intervals, indicating good repeatability regardless of the time interval between measures.

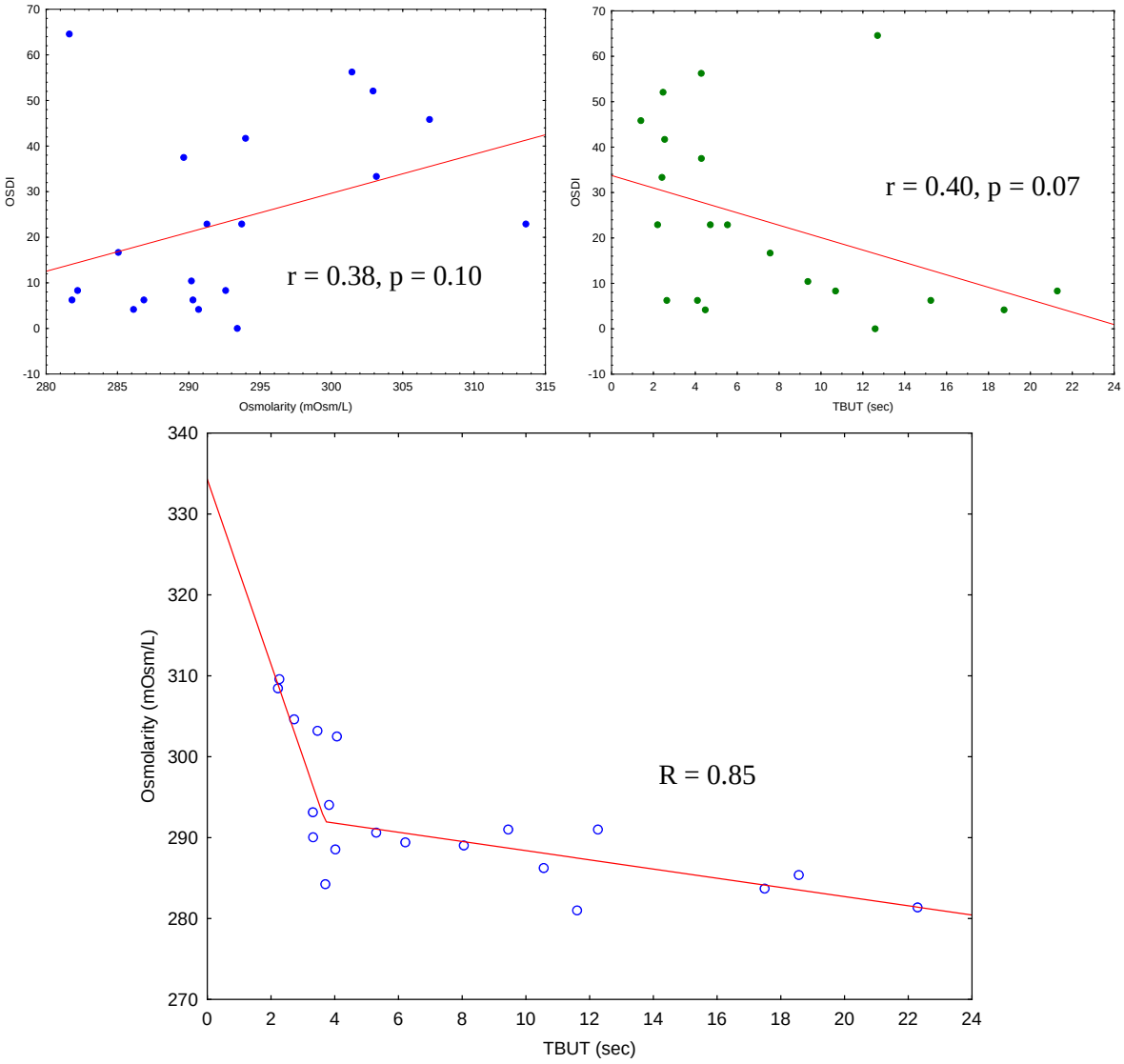


Figure 3-2. Graphical representation of relationships observed between OSDI scores, osmolarity and TBUT. Only TBUT and osmolarity showed a high degree of correlation (R = 0.85).

Phase II included eight males and 22 females with a mean age of 36.7 ± 15.1 years, and a range between 20 and 73. Mean *in vivo* osmolarity in the control and dry eye group was 287.7 ± 8.01 mOsm/L and 295.8 ± 12.5 mOsm/L respectively (p = 0.0058)[Table 3 – 1]. As observed in phase I, OSDI scores and TBUT were significantly different between the control group and the dry eye group

($p < 0.001$ for both variables). No significant differences were observed between left and right eye osmolarity ($p = 0.121$) or TBUT measures ($p = 0.724$). Interestingly, a significant difference was noted between consecutive measurements ($p < 0.001$), however when measurements were factored by group, no significant difference between measurements was observed ($p = 0.407$) [Figure 3 – 3]. A *post hoc* Tukey HSD test did show that the difference between the first measurement and the last measurement in both the control group and dry eye group bordered on significance ($0.05 < p < 0.054$ for both).

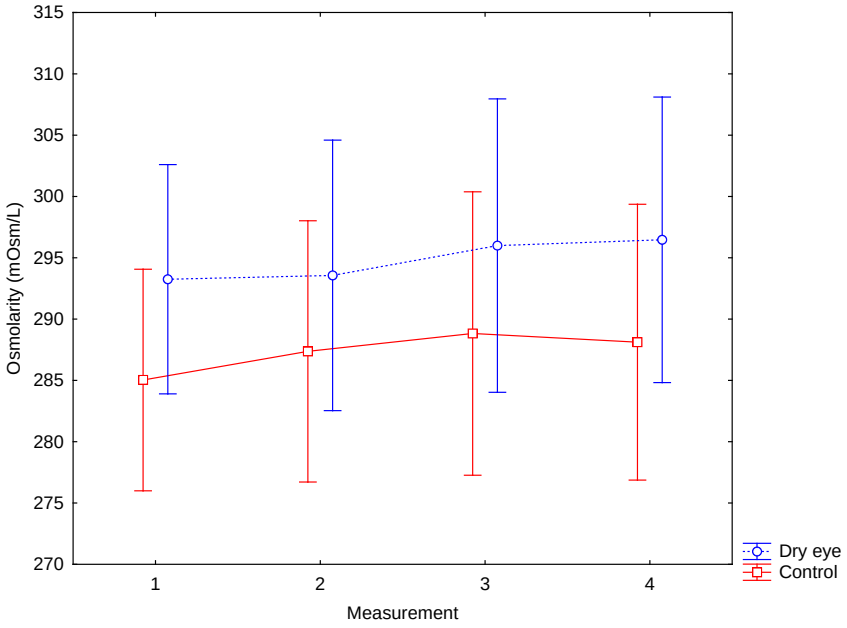


Figure 3-3. Mean osmolarity of each measurement for the control and dry eye group.

Relationships similar to those observed in phase I between osmolarity, TBUT and OSDI scores were also observed in phase II, although in phase II all were statistically significant, as indicated in Figure 3 – 4. Again, a bilinear function was fit to the data comparing TBUT and osmolarity, which demonstrated a significant linear relationship between the variables. The mean osmolarity of the tear film collected via capillary tube was 282.4 ± 9.8 mOsm/L in the control group and 291.1 ± 9.9 mOsm/L in the dry eye group ($p = 0.041$). Comparisons between measures taken *in*

vivo versus those with the capillary tube were significantly different in the control group (p = 0.006), but were not significantly different in the dry eye group (p = 0.10).

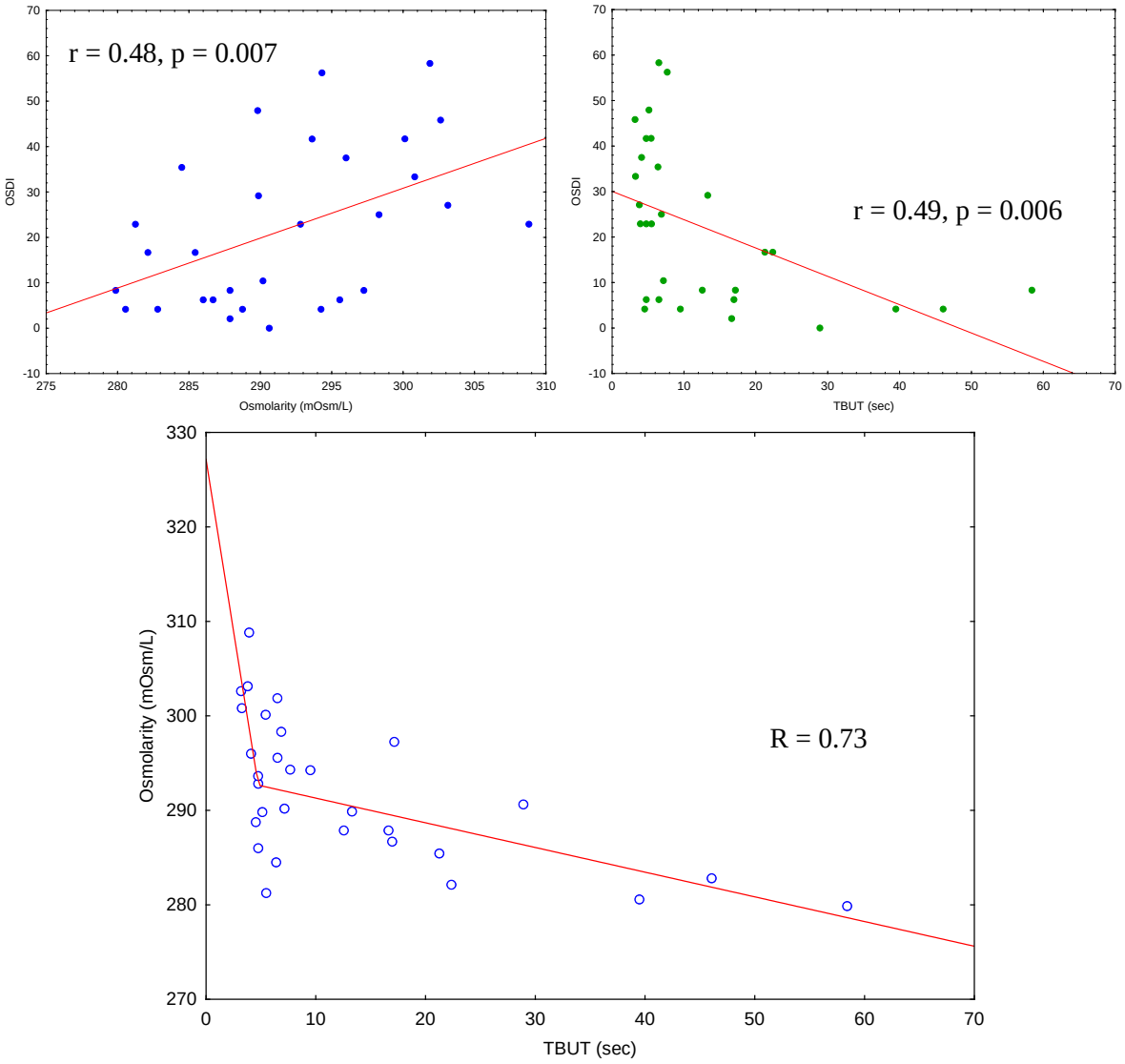


Figure 3-4. Relationships between OSDI, osmolarity, and TBUT in phase II. Only osmolarity and TBUT showed a high degree of correlation (R = 0.73), but all three were statistically significant.

3.5 Discussion

With the release of the results from the DEWS report, there has been a renewed interest in the use of osmolarity as a diagnostic marker of dry eye.[13] The limiting factors in the adoption of osmolarity measures in a clinical or research setting has always been a lack of instrumentation that is compact and efficient, and the large volume of tears required by most osmometers for analysis. The introduction of the TearLab overcomes many of these methodological issues, but very little is known about its performance in normal and dry eye populations.

There are only three independent reports that the author is aware of that present data gathered from the TearLab in normal and dry eye groups.[24-26] Tomlinson et al. compared osmolarity measures using the TearLab to those obtained with the Clifton nano-osmometer in 36 subjects.[26] Fifteen of the subjects were classified as dry eye based on non-invasive TBUT (NIBUT) of <10 seconds and being positive for symptoms of dry eye. They found TearLab mean osmolarity values of 308 ± 6.2 mOsm/L in the control group, and 321 ± 16.5 mOsm/L in the dry eye group, which correlated well with the results from the Clifton nano-osmometer ($r = 0.904$). A later study by Benelli aimed to use TearLab osmolarity measures as a method of charting the treatment efficacy of commercially-available lubricating eye drops in those suffering from symptoms of mild dry eye.[24] 60 participants were selected based on an OSDI score between 30 and 60, and a Schirmer test of <7mm after five minutes, then divided into three treatment groups. Mean osmolarity of each treatment group was 320.6 ± 2.0 mOsm/L, 320.9 ± 3.4 mOsm/L, and 321.9 ± 2.7 mOsm/L. The largest, and most exhaustive, study published thus far evaluated tear osmolarity measures using the TearLab in 200 subjects, and failed to demonstrate a difference between the control and dry eye group, with a mean osmolarity of 307.1 ± 11.3 mOsml/L and 308.9 ± 14.0 mOsm/L respectively.[25]

The mean osmolarity of either the control or dry eye groups in the above studies are starkly contrasted to those in the present study. At approximately 288 mOsm/L, the osmolarity in the control

group for both phase I and II is almost 20 mOsm/L lower than those outlined above. Mean osmolarity in the dry eye group, 297.8 ± 14.7 mOsm/L, is again approximately 20 mOsm/L lower than two of the three studies outlined above. Readings in both groups that are consistently 20 mOsm/L lower than other published observations may suggest an issue with the instrument used in the study. However, the TearLab was calibrated daily per the manufacturer's instructions with both an electronic chip card and saline calibration standards, and calibrated as expected each time.

A possible explanation for the low observed osmolarity, particularly in the dry eye group, may be the use of a questionnaire to differentiate dry eye and control subjects. Although symptomatology plays an important role in the diagnosis of dry eye, numerous studies have shown that symptoms weakly correlate with objective signs of dry eye.[19, 27-31] For example, Tuisku estimated correlations between OSDI and TBUT ($r = -0.32$), and OSDI and Schirmer's ($r = -0.47$), the latter proving to be significant ($p < 0.05$).[30] Conversely, another study was unable to show a significant relationship between Schirmer's and OSDI ($r = -0.182$, $p = 0.14$), but was able to show a relationship between OSDI and TBUT ($r = 0.296$, $p = 0.01$).[31] There is only one study that has specifically compared osmolarity measures to OSDI scores.[19] Using the Advanced Instruments osmometer, very little correlation between the two variables was observed in a group of 40 mild-moderate dry eye sufferers ($r = 0.033$). Despite the presence of relationships between signs and symptoms of dry eye, the available data would suggest that the correlation is weak and quite variable. Certainly, comparisons made between osmolarity and OSDI scores in this study support that statement, as correlation coefficients were 0.37 for phase I and 0.47 for phase II. Therefore, it may have been prudent to include additional objective tests to better categorize our subjects.

Multiple measurements are often used as a method for increasing accuracy and reducing the effects of the inherent variability of a given instrument. However, taking multiple samples, particularly in close succession, of the tear film may pose a problem as it may negatively influence its

natural state, and artificially inflate osmolarity readings. We were able to show that it is possible to safely collect four consecutive measurements, whether at 15 minute or 1 minute intervals, without significantly influencing osmolarity values in both dry eye and control subjects. It is notable that a gradual increase between successive measures was observed in the dry eye group using a one minute time interval (Figure 3 – 1), and it is likely that additional measures would cause a significant increase in osmolarity values. ICC values also indicated the instrument is highly repeatable when measures are taken at 15 minute or 1 minute intervals. Therefore, we recommend collecting no more than four samples from a given eye at 60-90 second intervals as a way to maximize accuracy and improve efficiency.

The DEWS subcommittee report on the classification of dry eye listed two core mechanisms as the driving force behind dry eye – tear hyperosomolarity and instability.[13] The hypothesis put forward was that in many cases of dry eye, tear film instability is the initiating event, and hyperosmolarity follows as a consequence. The strong relationship observed between TBUT, a commonly used method for estimating tear stability, and osmolarity in both phase I and phase II supports this theory. This also compliments earlier work by Liu et al. that provided indirect evidence of a link between hyperosmolarity and tear instability.[32] A second measured variable in the study that supports the link between osmolarity and instability of the tear film was the observed coefficient of variation (CV) values in the dry eye group. The manufacturer states that the instrument has a CV of 1.5%, a result that was independently confirmed elsewhere.[24] Accounting for the expected CV of the instrument, the control group has a CV of approximately 0.65%, or ± 2mOsm/L and the dry eye group has a CV of 1.9%, or ± 6mOsm/L. This indicates that measured osmolarity values in a dry eye population will fluctuate around the mean a considerable amount, while measures in a normal population will remain relatively constant. Oscillations in osmolarity values is very suggestive of a

tear film lacking stability, and therefore a better diagnostic criteria for dry eye may be an evaluation of an individual's CV versus their mean osmolarity.

Nelson and Wright evaluated potential sources of variation in tear osmolarity values in a small number of human subjects, with a focus on the effect of reflex tearing.[20] They used light as a source of irritation to induce a reflex tearing response in six normal subjects, and subsequently observed a 5% drop in osmolarity values, from 302 mOsm/L to 289 mOsm/L. Current osmometry techniques require the collection of 5-10 μL of tears using glass capillary tubes, a process that can be time-consuming and difficult in those suffering severe dry eye.[33] It is accepted, though has not been shown, that prolonged collection times and mechanical stimulation during collection will induce reflex tearing and lower osmolarity.[18] Hence, it is reasonable to postulate that a degree of reflex tearing is occurring when a glass capillary tube is used to collect tears. Although we suspect that mechanical stimulation of the ocular surface is the driving force behind the reflex tearing response, it is possible that the response is a result of emotional tearing due to anxiety or fear related to the collection process. One might argue that the use of a glass capillary tube is a more intimidating collection method, and might therefore induce a degree of tearing independent of the collection. Another factor that should also be considered when interpreting the results of phase I is the assumption that the osmolarity of reflex tears is lower than that of basal tears. There have been conflicting reports about the osmolarity of tears that are hypersecreted. Gilbard artificially manipulated the flow rate of the rabbit lacrimal gland, and found that an increased flow rate, or hypersecretion, led to a decrease in fluid osmolarity.[34] Other work with the rabbit lacrimal gland has shown the osmolarity to remain relatively constant despite flow rate.[35] Unfortunately, there is not a study that has specifically looked at changes in human tear fluid osmolarity with changes in flow rate, a factor that warrants further investigation.

The second phase of the present study aimed to determine if differences existed in osmolarity values when samples are obtained from the glass capillary tube versus the TearLab. The TearLab is thought to avoid the pitfalls of capillary tube collection, as it requires minimal contact of the ocular surface and collection times are virtually instantaneous due to the low volume required. The results showed a 5 mOsm/L drop in osmolarity values in those samples collected using the glass capillary tube, which does imply that a reflex response does occur despite the use of a carefully controlled collection method. The decrease in osmolarity may in fact have been greater that what was observed, as it is possible that evaporation of the sample during multiple transfers could have raised the osmolarity slightly. Regardless, this difference between collection methods was statistically significant in the control group ($p = 0.006$), but was not statistically significant in the dry eye group ($p = 0.10$).

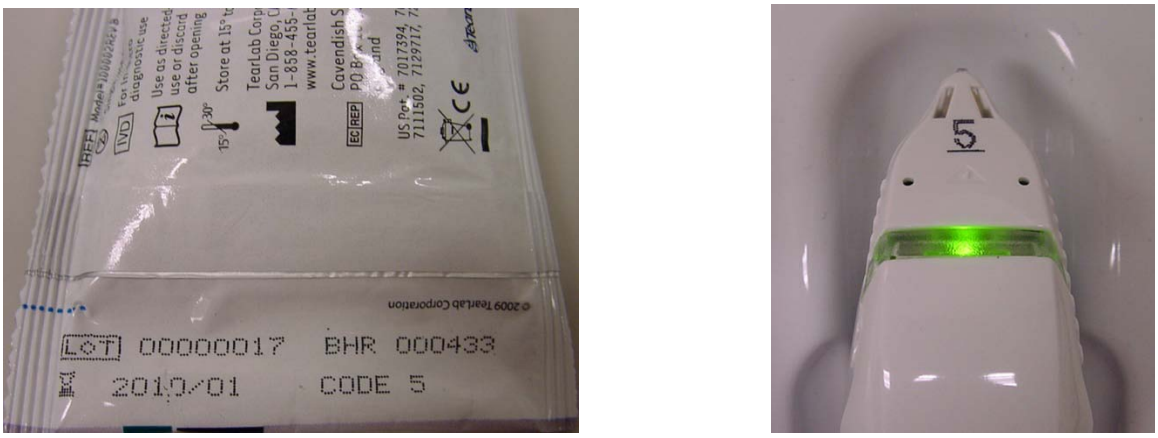
Difficulty in the collection process using the glass capillary tubes, particularly in the dry eye group, limits the conclusions that can deduced from the results. Although collection efficiency with the glass capillary tube was high in the control group at 96.7% (464 out of 480 possible measures), it was only 71.9% (345 out of 480 possible measures) in the dry eye group. This limits the conclusions that can be gleaned from the statistical analysis, and if a more complete dataset existed for the dry eye group, it is likely that the observed difference between methods would have been significant. It is also probable that the majority of the missing data is from those who have the most severe dry eye, as they are usually the hardest to collect tears from, which further biases the results. Although this is a limitation, it is also supportive of the technical ability of the TearLab to obtain a sample in those with severe dry eye or small tear volumes. A second unknown is the effect that the glass capillary tube itself has on osmolarity. It is possible that the capillary tube interacts with the tear components contributing to osmolarity, and this is a factor that warrants further study.

Overall, the present study has generated valuable data on the performance of the TearLab in a small subject population. It appears to be a repeatable method capable of collecting micro-volumes of tears from both normal and dry eye subjects, and in doing so, minimally perturbs the natural state of the tear film. In his extensive meta-analysis of literature relating to osmolarity, Tomlinson et al determined that using a cut-off osmolarity of 316 mOsm/L had a sensitivity of 59%, specificity of 94% and positive predictive value of 89% for the diagnosis of dry eye.[36] The observed mean osmolarity in the dry eye group for both phases of this study is so far below his suggested cut-off is of particular concern, and brings into question the ability of the TearLab to accurately identify those suffering from dry eye. This disparity may partially be explained by differences in instrumentation, as Tomlinson's analysis was primarily based on results from freezing-point depression and vapour pressure osmometers. Nonetheless, it is apparent that additional studies are needed to generate data on the ability of the TearLab to discriminate those with dry eye from normals before its use will be adopted into clinical practice.

Chapter 4
in vitro analysis of the performance of the TearLab nano-osmometer

4.1 Introduction

Although the previous chapter generated a significant amount of data on the performance of the TearLab in a research setting, it also raised a number of issues relating to the final results. Primarily, it was noted that the mean osmolarity in both the control (287.9 ± 7.5 mOsm/L) and dry eye (297.8 ± 14.7 mOsm/L) group was significantly lower than other studies using the same instrument have reported.[1-3] One of the major contributors to the result may have been the use of a subject's Ocular Surface Disease Index (OSDI) score as a grouping criteria. However, there may also have been instrument-specific factors that played a role. One of the most obvious variables with the TearLab is the effect of different chip cards on an individual measure, particularly since a new chip card is used for each measurement. All chip cards from a given lot have two unique identifiers, one being a batch history record (BHR), and the second being a code (Figure 4 – 1A). The code identified on the chip card (Figure 4 – 1B) must be entered into the instrument before a measure is made as a way of compensating for the known variability of the lot. It is currently unknown if using different chip card lots will have any influence on osmolarity readings. Therefore, one purpose of this study was to determine if differences exist between parallel osmolarity measures using chip cards with different lots/codes.



A **B**

Figure 4-1. (A) TearLab chip card package displaying the lot, batch history record (BHR), and code. (B) Chip card on the collection pen with the code presented.

Data from the previous chapter also demonstrated a lower osmolarity when tears were collected *in vivo* using the glass capillary tube (GCT), which was hypothesized to be due to induction of reflex tearing. However, the difference observed in osmolarity may be secondary to issues with the GCT method itself, such as a reduced uptake, or evacuation, of factors contributing to tear osmolarity (electrolytes and proteins). This concept is supported in a previous study by Jones *et al.*, in which two different methods for the collection of tear film were evaluated, and their relative efficacy of protein recovery compared.[4] The first method involved a GCT, and the second method used a porous polyester rod. They showed that although there was not a statistically significant difference in protein recovery, there was a trend towards reduced efficiency using the GCT. This would suggest that either the GCT has reduced uptake of the proteins, or the proteins are interacting within the GCT, preventing their evacuation out of the tube. Although proteins do not significantly contribute to osmolarity of the tear film, the electrolytes may interact similarly with the GCT. The second portion of this study evaluated the effect of using a GCT on fluid osmolarity.

4.2 Methods

The first portion of this study aimed to determine the effect of chip card lot/code on the measured osmolarity. To do so, two different control solutions of known osmolarity were used in combination with three different chip card batches (Code 3, 5 and 10). The associated lot and batch history record (BHR) for each chip card batch is shown in table 4 – 1.

Code	Lot	BHR
3	14	08-0354
5	17	433
10	29	675

Table 4-1. Description of lot and batch history record (BHR) for the three chip cards used throughout the experiment.

The two solutions used were the standard calibration controls for the TearLab, consisting of a normal and a high control. The normal control has an expected osmolarity of 292 ± 12 mOsm/L and the high control is expected to measure 338 ± 15 mOsm/L. Six chip cards were used from each batch to measure the same control, for a total of 18 measures on the normal control and 18 measures on the high control. The order in which each chip card was used was randomized.

The second portion of the experiment utilized three different control solutions to evaluate the effect of collection method on tear osmolarity. Two of the solutions were the control solutions provided by TearLab for daily calibration of the instrument outlined above, and the other was a contrived artificial tear solution (ATS) of known osmolarity. The ATS (Bionostics, Inc. Devens, MA, USA) was comprised of a complex mixture of ions, proteins, lipids and metabolites that mimicked the human tear matrix and had a known osmolarity of 294 mOsm/L. Sample size was determined using the observed mean and standard deviation obtained from the *in vivo* portion of Chapter 3 and setting the statistical power to 0.50. This resulted in a sample size of 18, meaning that for each control

solution, 18 measures would be taken using the TearLab, and 18 taken using the glass capillary tube. For each control, three different vials were used, with each vial being from the same lot, to eliminate lot-to-lot variation. Additionally, to minimize evaporative effects, vials were opened and sequentially measured 6 times with each method before being discarded.

The technique used for both portions of the experiment to measure osmolarity with the TearLab was relatively quick and simple. For each new chip card, the collection tip was carefully inserted into the vial opening to collect the sample, and then returned to the docking station for completion of the measure. The general technique used to obtain and process a sample of solution using the GCT was outlined previously (Chapter 3), but was slightly modified to fit within the constraints of the current experiment. In this case, the GCT was inserted into the control vial, whereupon 5µL of solution was collected. The contents of the GCT were immediately transferred to a 0.1mL PCR tube (Axygen Scientific Inc., Union City, CA, USA) and spun down for 7 seconds at approximately 6500rpms, then placed on ice. 500nL of the sample was aliquoted onto a clean 1" square of Parafilm (Pechiney Plastic Packaging Company, Chicago, IL, USA) and immediately collected by placing the collection tip of the TearLab into the dispensed sample.

4.3 Statistical analysis

All gathered data was transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) and imported to Statistica version 7 (Statsoft, Tulsa, OK, USA) for analysis. A one-way ANOVA was used to evaluate differences between batches, followed by post hoc analysis to further elucidate where any differences lay. A t-test for independent samples was used to compare osmolarity of samples collected directly with the TearLab or with the GCT. A repeated-measures ANOVA was also used to evaluate the impact of various factors, including control osmolarity and the use of different vials, on mean osmolarity. For all analyses, a p-value of less than 0.05 was considered statistically significant.

4.4 Results

The ANOVA of batch effects of the chips resulted in significant differences whether the 292 mOsm/L control ($p < 0.001$ or the 338 mOsm/L control ($p < 0.001$ was used (Figure 4 – 2A and 4 – 2B respectively). Tukey HSD determined that regardless of control osmolarity, batch 10 was significantly different than batch 3 or 5 ($p < 0.002$), while the mean osmolarity of those batches were statistically similar ($p > 0.78$).

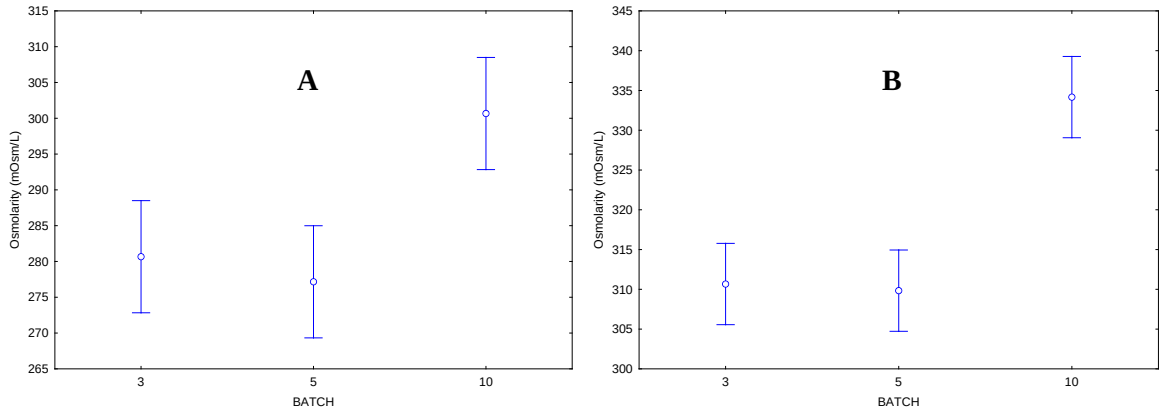


Figure 4-2. The results of the analysis of batch effects on mean osmolarity of the 292 mOsm/L control solution (A) and 338 mOsm/L solution (B), $p < 0.001$ in both cases.

Mean osmolarity of those samples taken directly from the vial was 295.8 ± 19.22 mOsm/L, and 300.3 ± 18.9 mOsm/L for those that were first collected via capillary tube, a difference which was not statistically significant ($p = 0.23$). Statistically significant differences were observed between the mean osmolarity of the three controls ($p < 0.001$), and this difference was still present when one factored in whether the control was measured with the TearLab or with the capillary tube ($p = 0.003$). However, *post hoc* analysis revealed that no differences were present between measurement methods for the 292 and 338 control, but were present when the ATS was used (Figure 4 – 3).

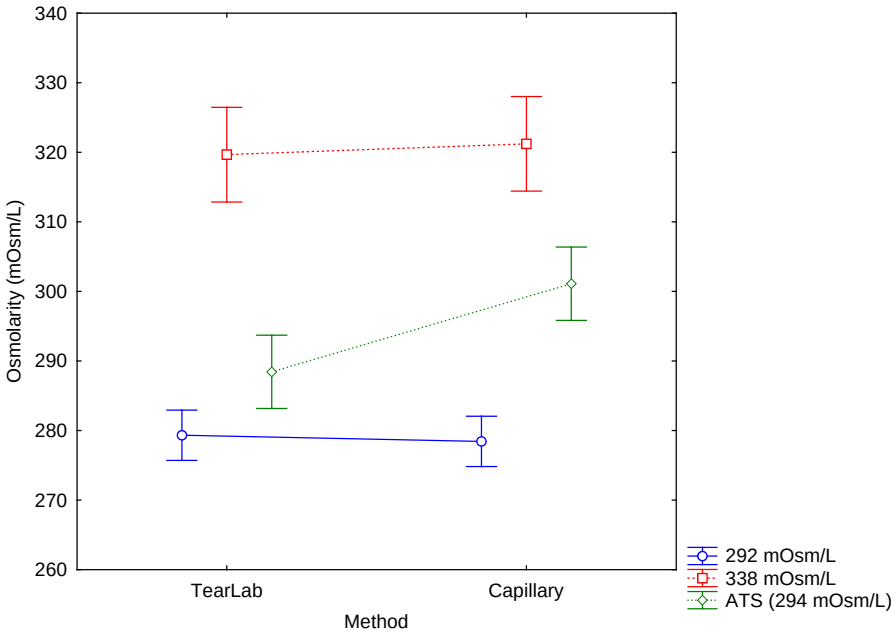


Figure 4-3. Comparison of mean osmolarity for the three controls sampled either directly by the TearLab or by the glass capillary tube.

When comparing the three vials used in each control group, differences were again noted amongst the ATS group, with all three vials measuring significantly different from one another (Figure 4 – 4). No differences were present between vials in the 292 mOsm/L and 338 mOsm/L control groups.

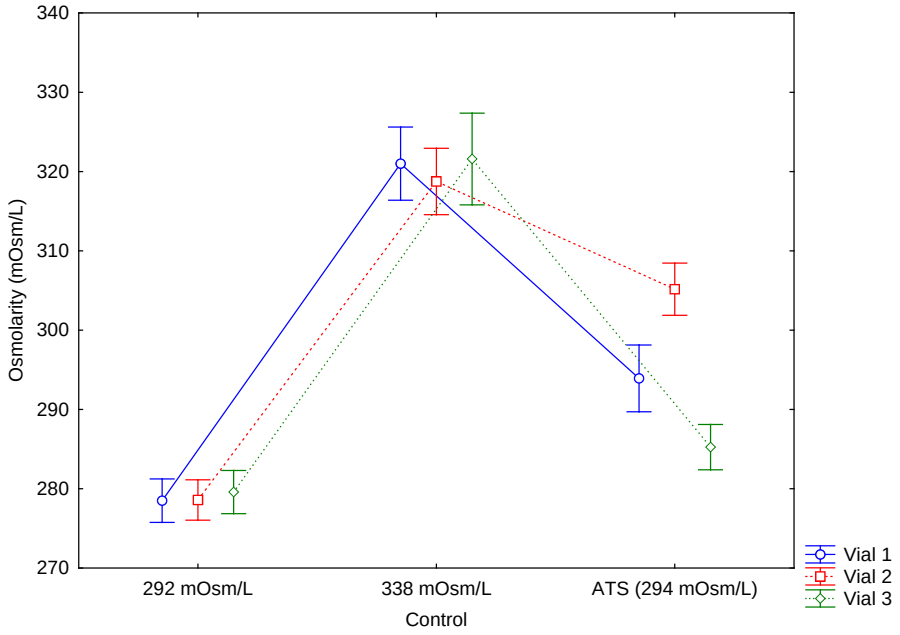


Figure 4-4. Mean osmolarity of each of the three vials used as a control.

4.5 Discussion

Although this was a relatively simple *in vitro* experiment, it has yielded unique insight into the functioning of the TearLab. The first portion of the experiment demonstrated that different chip card codes/lots can have a significant influence on measured osmolarity. For the 292/338 mOsm/L controls, Code 10 chip cards had a mean osmolarity of 300.7/334.2 mOsm/L, significantly higher than both code 3 (280.7/310.7 mOsm/L) and 5 (277.2/309.8 mOsm/L) chip cards. It is particularly worrisome that a 20 mOsm/L difference between individual lots existed, as a 20 mOsm/L difference can easily lead to either an over- or under-diagnosis of dry eye. Also of concern is the difference between the observed and expected osmolarity of the calibration controls. TearLab expects a deviation of ±12 mOsm/L in the case of the 292 control, and ±15 mOsm/L in the case of the 338 control. For the 292 control, only lot 3 and 10 would have fallen within the expected range and for the 338 control, only lot 10 was within the expected range.

The second portion of the experiment showed that although a statistically significant difference between collecting directly with the TearLab or with the GCT did not exist, the GCT appeared to nonetheless measurably influence osmolarity. In contrast to the previous *in vivo* study, which observed a 5 mOsm/L decrease in osmolarity, the present study demonstrated a 5 mOsm/L increase in osmolarity. This increase is likely attributable to evaporative effects, as the fluid was transferred multiple times before being measured on the TearLab. A surprising result was the significant difference in ATS osmolarity as measured with the GCT versus the TearLab. The primary purpose of the lipid layer in the human tear film is to form a barrier between the aqueous portion and the surrounding environment, thereby minimizing evaporation and maintaining osmolarity.[5] Therefore, one would expect that the lipids present in the ATS would perform the same function, and negate any evaporative effects that would occur during fluid transfer. This did not appear to be the case, as the osmolarity was significantly higher when measured with the GCT.

One possible explanation for the increased osmolarity may have been the collection technique used to gather the sample using the GCT. In the ATS, the hydrophobic nature of lipids would likely keep them on the surface of the solution. When the ATS was collected from its storage vial, the GCT was placed down into the solution, and it is possible that because collection was taking place well below the surface, very few lipids were collected. Without the lipid layer present, the aqueous portion was able to evaporate and concurrently increase osmolarity of the ATS.

As noted earlier, both control solutions have a fairly large range of expected osmolarity and it is possible that this range is secondary to variations in the manufacturing process of the controls. Figure 4 – 4 clearly shows no significant difference observed between the three vials used for the 292 and 338 mOsm/L controls, which suggests that the range may in fact be due to variations in the chip cards. Differences were present between the three contrived ATS, but this is perhaps less surprising given the complexity of the solutions.

The primary limitation of the present study was the low sample size, particularly for the portion that evaluated batch effects. This is a reflection of the significant cost involved for each chip card, estimated to be $15-25 per card. A small sample size lowered the statistical power, but the effect size appears to be significant enough to stand as being a true observation. A second limitation is the unknown effect of time on the viability of the chip cards. The oldest chip cards, batches 3 and 5, were received approximately 10 months before the present study was carried out, while the newest of the chip cards, batch 10, was received only 5 months prior. Coincidentally, it was batch 3 and 5 that were significantly different from batch 10; however, they were not different from one another. Chip cards were used well in advance of their stated expiry date, and all were stored in temperature and humidity controlled environments with no exposure to sunlight or electrical devices that could interfere with their functioning. This raises the possibility that there is a temporal degradation in the chip card's measurement abilities, and warrants further investigation.

In conclusion, there appears to be some technical issues with the operation of the TearLab, the majority of which are related to the chip cards. As the instrument is relatively new and the large-scale manufacturing process of the chip cards is in its infancy, it is hoped that many of these concerns will be addressed in subsequent generations of the instrument and chip card.

Chapter 5

Evaluation of the relationship between tear film osmolarity, tear meniscus height and symptoms of dry eye

5.1 Introduction

Tear osmolarity has been cited as the 'gold standard' for the objective diagnosis of dry eye,[1] and has been attributed to many of the signs and symptoms observed throughout the natural history of the disease process.[2, 3] Gilbard, an early pioneer in tear osmolarity research, postulated in 1986 that hyperosmolarity was a function of either increased evaporation rates of the tear film from the ocular surface, or decreased production of the aqueous layer by the lacrimal gland.[4] He also suggested a third possibility, based on work undertaken with rabbit lacrimal gland ducts,[5] which is that the osmolarity of lacrimal gland fluid itself increases, although no human study has supported this concept. His postulates have persisted, and the Dry Eye Workshop document pertaining to the definition of dry eye stated "The major causes of tear hyperosmolarity are reduced aqueous tear flow, resulting from lacrimal failure, and/or increased evaporation from the tear film."[6] A distinct implication of the DEWS document and Gilbard's work is that tear film osmolarity may be volume-dependent.

Tear meniscus height (TMH) has been proposed as a clinically useful estimate of the volume of the tear film.[7] If measured without the use of fluorescein as an indicator it is non-invasive, and it has relatively high sensitivity and specificity for the diagnosis of dry eye.[8] For the aforementioned reasons, Fourier-domain optical coherence tomography (FD-OCT) is ideally suited to measure TMH, and its high resolution and integrated software should make the measurement process easier and more accurate.

The primary objective of this study was to determine if a relationship exists between osmolarity and TMH measures in a mild-moderate dry eye population. A secondary objective was whether various subjective ratings of dry eye symptoms corresponded to either osmolarity or meniscus height.

5.2 Methods

The study was cleared by the Office of Research Ethics at the University of Waterloo and adhered to all tenets of the Declaration of Helsinki. Forty-five subjects were recruited for participation in the study and the primary inclusion criterion was a score of ≥ 20 on the Ocular Surface Disease Index (OSDI). Subjects were excluded from participation if they had undergone refractive surgery or if conjunctivochalasis was present. Subjects were asked to refrain from contact lens wear 24 hours before their visit and to avoid the use of artificial tear solutions for six hours prior to their visit. After receiving a subject's informed consent, a five-question linear visual analogue scale (VAS) designed to subjectively rate common symptoms of dry eye was completed. The five symptoms evaluated were comfort, dryness, burning, grittiness and clarity of their vision, and a rating was given for each eye on a interval scale of 0 – 100 (0 being severely symptomatic and 100 representing no symptoms).

Tear meniscus height measurements were then obtained on each eye in triplicate using the RTVue-100 Fourier-domain optical coherence tomographer (Optovue, Freemont, CA, USA). The CAM-S (Cornea/Anterior Module – Short) lens was used for all measurements, using the default settings of the instrument. The measurement was taken at the six o'clock position of the cornea, and the 2.0mm scan beam was aligned so that its midway point bisected the margin of the lower lid. Subjects were asked to maintain their habitual blink frequency, and images were captured immediately after a blink. Image analysis was conducted using the integrated software, and the

meniscus height was defined as the vertical difference between the point at which the tail of the meniscus intersected with the cornea superiorly and the lower lid inferiorly.

At the conclusion of the study visit, four tear samples were taken from each eye of the subject using the TearLab nano-osmometer (TearLab Corp., San Diego, CA, USA). To collect the tears, subjects were instructed to direct their gaze superonasally, whereupon the tip of the chip card was gently placed into the tear meniscus near the lateral canthus. Care was taken to minimize contact with the ocular surface and avoid a reflex tearing response. After a successful collection, the pen and chip card were immediately returned to the docking station for analysis.

5.3 Statistical analysis

All data was entered into Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) and subsequently exported to Statistica version 7 (Statsoft, Tulsa, OK, USA) for analysis. Relationships between age, TMH, OSDI and osmolarity values were evaluated using Pearson's correlation co-efficient, with $p > 0.60$ considered a high strength of agreement. A repeated measures ANOVA was used to evaluate any differences in TMH, osmolarity and linear VAS values for the right and left eye. Subjects were then stratified into four groups based on their mean osmolarity and TMH values of the right eye (Table 5 – 1).

Group	**Osmolarity (mOsm/L)**	**TMH (mm)**
Low/Low	< 300	< 0.3
Low/High	< 300	> 0.3
High/Low	> 300	< 0.3
High/High	> 300	> 0.3

Table 5-1. Breakdown of grouping criteria based on tear osmolarity and tear meniscus height.

The cut-off value of 300 mOsm/L was selected for osmolarity based on the approximate mean osmolarity of the dry eye group observed in Chapter 3. The TMH cut-off of 0.3mm was based on the work of Ibrahim et al., who determined that a TMH value of 0.3mm had a sensitivity and specificity of 67% and 81% respectively for the diagnosis of dry eye.[9] One-way ANOVAs were used to evaluate differences in objective and subjective data between the four groups, and a *post hoc* Tukey HSD test was used as appropriate to determine where, if any, differences existed. A p-value of < 0.05 was considered statistically significant.

5.4 Results

Nine of the subjects were male, and 36 were female, and the mean age of all subjects was 47 (range 17 – 79). Mean TMH for the right eye was 0.281 ± 0.123mm and for the left eye was 0.297 ± 0.130mm, a difference that was not statistically significant ($p = 0.34$). Osmolarity values for the right and left eye, 302.9 ± 16.4 mOsm/L and 301.2 ± 14.8 mOsm/L respectively, were also not significantly different ($p = 0.21$). Pearson's correlation coefficient did not describe a significant relationship between TMH and osmolarity ($r = -0.14$, $p = 0.36$)[Figure 5 – 1]. No difference was observed between left and right eyes for linear VAS values ($p = 0.94$). Pearson's correlation coefficients were unable to demonstrate relationships between age, OSDI scores, osmolarity or TMH measures, although the relationship between OSDI score and osmolarity bordered on statistical significance ($p = 0.057$).

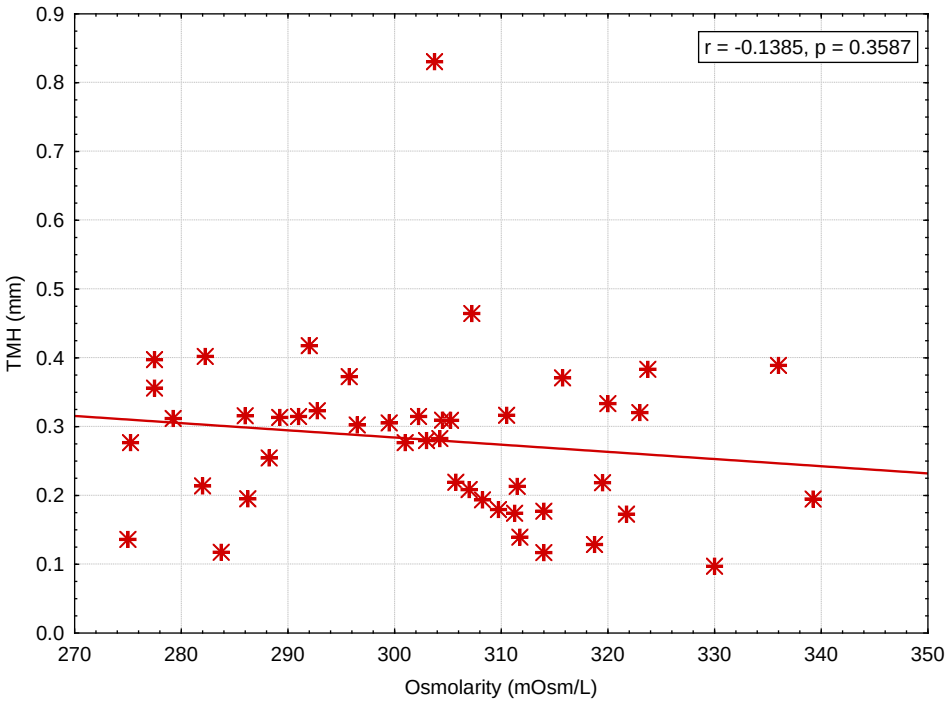


Figure 5-1. Plot of osmolarity versus TMH with the line of best fit showing a trend of decreased TMH as tear osmolarity increases.

No difference was observed between each of the five symptoms rated as part of the linear VAS ($p = 0.99$), nor were any of the symptoms statistically different from one another when the grouping criterion was taken into consideration ($p > 0.60$). Taking the mean value of all symptoms rated, those in the Low/Low group had the lowest mean value, with an average rating of 54.5. Those in the High/High group had the highest average rating, at 61.3. A significant difference was noted in age between groups, particularly between the Low/Low group and the High/Low group ($p = 0.036$)[Figure 5 – 2]. As expected, the TMH and osmolarity was also significantly different between groups ($p < 0.000$).

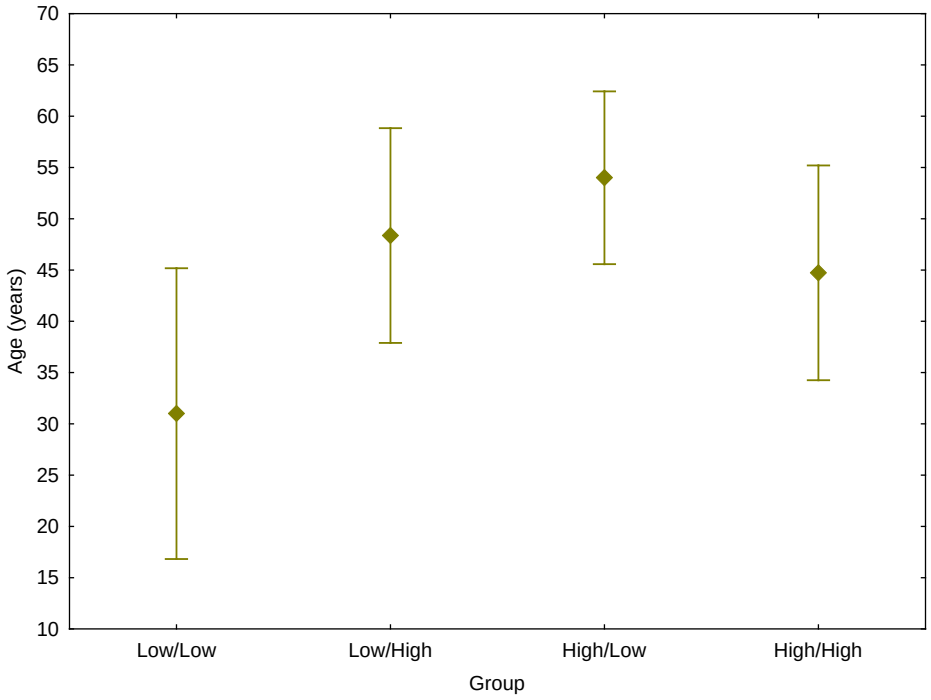


Figure 5-2. Mean age and 95% CI of subjects in each group.

5.5 Discussion

There are a number of studies that have evaluated tear meniscus height and tear dynamics in a dry eye population using either a custom-built OCT[8, 10, 11] or a commercially available anterior-segment OCT (AS-OCT).[9, 12] Using a custom-built real-time OCT, Shen et al. found a mean TMH of 0.196 ± 0.023mm in the right eye and 0.190 ± 0.024mm in the left eye in a group of 48 subjects with aqueous-deficient dry eye (ADDE).[8] Yuan et al. used the same instrument setup and similar grouping criteria to measure the lower TMH before, during and after the interblink period, and observed a mean TMH of 0.237 ± 0.100mm immediately following a blink. In close agreement with Yuan et al.'s measure of lower TMH, Ibrahim et al. used a commercially-available Visante AS-OCT to image the upper and lower TMH of 24 dry eye subjects, and found a mean TMH of 0.25 ± 0.08mm.[9]

The high mean (0.290mm) and standard deviation (0.126mm) of the TMH observed in this study versus other studies is likely due to two factors: grouping criteria and instrument differences.

Chen et al.,[10] Shen et al.,[8] and Yuan et al.[11] all used subjects suffering from ADDE, a group that one would expect to have very low tear volumes and hence, low TMH. By using the OSDI as our grouping criteria we were much less specific, and therefore have included those with ADDE and those with evaporative dry eye (EDE). It has been suggested that EDE disease is characterized by a normal-high tear volume as a result of a compensatory lacrimal reflex response to the compromised meibomian glands.[13] This will subsequently lead to a higher meniscus height, and may account for the higher mean and standard deviation observed. Geographical and racial differences may also influence results, made apparent by the Shen et al.(carried out in Miami, FL, USA) and Yuan et al.(carried out in Wenzhou, Zhejiang, China) studies that used the same grouping criteria and instrument, but had very different results.[8, 11] Instrument specific differences have been demonstrated in comparative studies between various types of commercially-available OCTs.[14, 15] Variations in instrument-specific algorithms for determining image parameters have been suggested as the cause of such differences, in addition to the differences in resolution of the various instruments. Unfortunately, there is no published study that the author is aware of that has evaluated the TMH measurements in a dry eye population using the RTVue-100. Therefore, some caution must be taken when comparing the results of our study to those outlined above.

Tear osmolarity values observed in the present study, at 302.9 ± 16.4 mOsm/L for the right eye and 301.2 ± 14.8 mOsm/L for the left eye, remain well-below what one might expect based on the recruitment of mild-moderate dry eye sufferers. Other studies that have used the TearLab have found mean osmolarity values in the dry eye group in the range of 309 – 321 mOsm/L.[16-18] As noted and expanded upon in Chapter 3, the lower than expected values might suggest that there is an issue with our instrument or the method we used to select our study population. If the TearLab used in the study is not functioning in the same manner as other devices it may explain the lack of an association between TMH and osmolarity. However, one other study that looked to correlate TMH and

osmolarity in those with nasolacrimal duct obstruction failed to show any association between the two variables.[19]

The concept of associating specific symptoms with various ocular surface abnormalities is certainly not new. Varikooty used psychophysical methods to demonstrate various mechanical (scratchy, dry), chemical (burning, stinging) and itch symptoms throughout the interblink period.[20] He was able to show that immediately after a blink, mechanical (38%), chemical (33%) and itch (29%) symptoms were all experienced with approximately equal frequency. However, near the end of the interblink period, symptomatology had shifted and 91% of symptoms were chemical, 9% mechanical and no itch was perceived. A more recent study by Liu et al. used visual analogue scales (VAS) to rate the overall intensity of discomfort of hyperosmolar solutions between 300 and 1000 mOsm/kg that were instilled in the eye.[21] One drop of either a sodium chloride or sucrose solution was placed into one eye, followed immediately by each subject rating burning, stinging, irritation, pricking and cooling on the VAS. They were able to show that as the osmolarity of the instilled solution increased, the intensity of symptoms similarly increased in a linear fashion. A statistically significant difference was demonstrated between symptoms, as irritation, burning and stinging were reported at high levels, and cooling was reported the least.

In the present study, dryness and grittiness had the lowest values, indicating they were perceived to be the most intense of the symptoms, although they were not significantly different than the other symptoms evaluated. We also failed to demonstrate a symptom that appeared to be more prevalent within a single group. One reason may be an inherent issue of using VAS, namely the non-linearity of such scales. In a comparison of the consistency between a VAS and a discrete scale designed to measure the same variable, subjects were asked to rate pain on the VAS and a 7-point scale with descriptive wording tied to each value (0 being no pain, 6 being unbearable pain).[22] A high degree of variability and overlap was found between VAS scores and each category of the discrete

scale, which suggests that scores are individual and should not be used for group descriptions. A second reason may have been a lack of training of subjects to accurately quantify their symptoms. In the study by Liu et al. outlined above, all subjects had previous psychophysical training to detect small differences in corneal sensation before they participated.[21] Training of subjects may have eliminated some of the known variability of the VAS and improved the responses.

Bron et al. have recently published some work on the pathophysiology and hypothesized key mechanisms behind dry eye.[13] This paper summarized current literature to characterize ADDE and EDE, and also described a late-stage hybrid form, wherein ADDE would take on some of the characteristics of EDE and vice versa.[13] The breakdown of mean age by group, as seen in Figure 2, may yield some insight into the temporal development of dry eye and support some of the suppositions put forward by Bron.[13] It is possible that the Low/Low group, which had the lowest mean age at 31, represents an early-ADDE group, as they have a low meniscus, but not the high osmolarity that Bron believed would be present. A low osmolarity could be maintained within this group due to a stable lipid layer, and various papers have established the increased lipid stability and volume and decreased evaporation that occurs in younger people.[23-25] Interestingly, the Low/Low group was also the most symptomatic, although only slightly so, a finding which may be due to the increased corneal sensitivity noted in those who are younger.[26] The Low/High and High/High group likely represents those with EDE disease based on mean age of the two groups (approximately 45 years) and the high meniscus. The increased age makes this group more likely to undergo changes to the meibomian glands that result in decreased tear stability and increased evaporation.[23-25] The higher meniscus is secondary to an increased sensory drive from the ocular surface in response to local alterations of osmolarity that results in an increased lacrimal response. Finally, I propose that the final group, High/Low, represents the hybrid form suggested by Bron et al., and is the end-stage of the disease.[13] For those in the High/Low group who began with EDE, a chronic dry eye state has induced

corneal hyposensitivity,[27, 28] and reduced the sensory drive to generate a compensatory reflex response. This results in a tear film exhibiting a high osmolarity and low tear volume. For those in the High/Low group who began with ADDE, the natural decline of meibomian gland secretions has marginally reduced tear stability, but coupled with the already present loss of lacrimal flow, results in a low volume, high osmolarity state.

Although the present experiment could not determine the existence of a relationship between osmolarity and TMH, or identify predominant symptoms within distinct dry eye groups, it has made some interesting observations regarding the possible temporal development of dry eye disease and its sub-types. It is important to note that this study was not designed to test a hypothesis regarding the temporal evolution of dry eye disease, and future work is needed to elucidate whether these observations would stand in a more rigorously designed study.

Chapter 6
Summary

The aim of this thesis was to use two new commercially-available devices, the RTVue-100 Spectral/Fourier-domain optical coherence tomographer (SOCT) and the TearLab nano-osmometer, to generate data on commonly used objective parameters for the diagnosis of dry eye.

The experimental portion of Chapter 2 measured tear meniscus height (TMH) in 50 subjects and demonstrated the significant differences that existed between a time-domain optical coherence tomographer (TOCT) and SOCT when measuring TMH. One factor attributed to the observed difference was the dewarping algorithm that the SOCT employs for proper imaging of the anterior segment, a feature that the TOCT does not use. The SOCT also had a larger measured TMH than the TOCT, and may be a result of the increased resolution of SOCT, which made delineation of the (particularly the upper meniscus) meniscus much easier. Despite the observed differences, the SOCT's standardized integrated analysis software made TMH measurements easier and allows for consistency between multiple centres that are using TMH as a variable, and we therefore advocate its use.

Chapter 3 was a two-phase study that presented preliminary data on the functionality of the TearLab nano-osmometer. The primary objective of the study was to generate data using the TearLab in both a normal and dry eye population. Mean osmolarity for normals was approximately 288 mOsm/L and for the dry eye group was approximately 297 mOsm/L and although these two groups proved to be significantly different from one another, the results are substantially lower than other published studies, including those using the TearLab.[1-7] The results do bring into question the accuracy of the instrument, and provided the impetus for the study performed in Chapter 4.

One of the major barriers to the acceptance of tear osmolarity as a clinical test has been the difficulty of collecting a tear sample while minimally disturbing the tear film. With this in mind, a second objective of the study in Chapter 3 was to determine whether any difference in osmolarity values would be observed whether tear collection was performed using a glass capillary tube or the TearLab. The glass capillary tube measures were 5 mOsm/L lower than those collected by the TearLab, a difference that was statistically significant. This suggests that despite careful collection practices, the glass capillary tube induces a degree of reflex tearing, which artificially lowers osmolarity. A significant difference in osmolarity between collection techniques was not demonstrated in the dry eye group, owing to difficulty in successfully collecting tears using the glass capillary tube. The ability of the TearLab to collect tears in those with moderate dry eye, and possibly a low tear volume, highlights one of the major technical advantages of the TearLab over other collection techniques. The final objective of Chapter 3 was to evaluate whether a short (1 minute) or long (15 minute) interval between multiple collections would yield a reproducible result. The results showed high repeatability (ICC values greater than 0.89) and minor influence on tear osmolarity values whether collections were performed at 1 or 15 minute intervals. To maximize efficiency, we concluded that a maximum of four tear collections could take place spaced 60-90 seconds apart without negatively influencing tear osmolarity. Perhaps the most interesting finding of the third chapter was the strong relationship between tear break-up time (TBUT), an established measure of tear stability, and tear osmolarity. Tear stability and osmolarity are inextricably linked in the current literature as core mechanisms in dry eye development, despite very little objective evidence associating the two; however, this experimental discovery supports and adds justification to that connection.

Chapter 4 used *in vitro* methods to elaborate on findings from Chapter 3, particularly the low osmolarity observed in both groups. As each measure is performed using a new chip card, an obvious

source of error may have been differences between chip cards. The results of Chapter 4 supported that hypothesis, as significant differences were noted between three different batches of chip cards that were used throughout the study conducted in Chapter 3. A second purpose of Chapter 4 was to determine if the glass capillary tube collection method was responsible for the decrease in osmolarity detected in Chapter 3. Calibration standards and a contrived artificial tear were employed to establish that using a glass capillary tube does not alter solution osmolarity, validating the results of Chapter 3.

Chapter 5 brought together aspects of previous chapters to answer two experimental questions: is osmolarity merely a function of tear volume, and are there any predominant symptoms in those with tear dysfunction? 45 subjects with mild-moderate dry eye had TMH, osmolarity and linear visual analogue scales measured. No significant relationship could be observed between any of the variables. The subjects were also placed into sub-groups for further analysis based on their TMH and osmolarity results. The most intriguing finding of the secondary analysis was the mean age of each sub-group, which fit nicely into the model proposed by Bron for the development of aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE).[8]

Chapter 2 and 4 also focused on the correlation of osmolarity with various objective and subjective measures of dry eye. Chapter 2 managed to establish a strong relationship between osmolarity measures and tear-break up time, and also provided some evidence of a weak, but statistically significant, relationship between osmolarity and OSDI scores. Chapter 4 was unable to demonstrate a significant relationship between specific dry eye symptoms and osmolarity or TMH. The inability to correlate signs and symptoms of dry eye is not new,[9, 10] but it does serve to remind that the absence of a relationship does not imply the absence of dry eye. As suggested elsewhere[11] and supported within this thesis, multiple diagnostic tests are needed for the accurate diagnosis of dry eye.

Although this thesis primarily related to techniques for the objective analysis of the tear film, it also served to evaluate the role of technology in research and clinical practice. An exponential

increase in the fundamental understanding of the function/structure of the human eye has driven the development of a wealth of instrumentation for its measurement and analysis. With the introduction of any new device, it is important that three aspects of the instrument are established: utility, accuracy, and precision. From a clinical perspective, an instrument has utility if it can assist in the management of a given condition, and determine whether or not treatment needs to be initiated. Accuracy is determined through the calibration of the instrument and comparison to accepted standards, and certainly the importance of calibration was established in Chapter 2. Finally, the use of multiple measures is employed to determine instrument precision, and this was a particular topic of interest throughout Chapter 3 and 4 in relation to the TearLab.

As an instrument for the analysis of tear meniscus height, the RTVue-100 performed admirably. The high-resolution images it produces make for easy determination of the meniscus borders, and the user-friendly integrated software allows for analysis of captured images. Additionally, it is capable of obtaining images quickly, and non-invasively, which reduces any effect it may have on the tear film. The interchangeable lenses (CAM-S and CAM-L) allow the RTVue-100 to image anterior-segment structures at different resolutions, giving the instrument greater flexibility. Despite the two lenses allowing for different scan lengths to be used, the biggest downside of this instrument is the maximum scan length of 6mm, meaning that only the upper or lower meniscus can be imaged at a given time. An improvement would be the ability to image a larger area of the ocular surface, closer to the 16mm scan length of the Visante device. Regardless, the implementation of the RTVue-100 in clinical or research practice is advocated.

Despite the reported importance as a single objective test with high sensitivity,[7] osmolarity measures have failed to be introduced into clinical practice due to a lack of instrumentation that is compact, user-friendly, cost-efficient and minimally-invasive. The TearLab appears to have overcome several of these limitations and throughout the various studies proved itself to be incredibly adept at

the collection and efficient analysis of tear osmolarity. Its novel use of lab-on-a-chip technology for the measurement of osmolarity is a significant step forward and positions the device for acceptance as a diagnostic tool. However, some technical issues remain and chief among those was the variance of measures due to different chip cards that was demonstrated as part of Chapter 4. A second issue with the device was the wide range of values considered acceptable for calibration purposes. Normal calibration solutions are expected to measure 292 ± 12 mOsm/L and high calibration solutions are expected to measure 338 ± 15 mOsm/L. When one considers the minute difference between a normal osmolarity (302 mOsm/L) and dry eye osmolarity (316 mOsm/L),[7] the TearLab may not have the sensitivity required to differentiate the two. These factors, in addition to the range of TearLab osmolarity values reported in the literature,[1, 2, 12] clearly demonstrates that further work is required to confirm its value in routine clinical practice.

Appendix A

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Chapter 1

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