The Development of a Validated Clinically Meaningful Endpoint for

the Evaluation of Tear Film Stability as a Measure of Ocular Surface

Protection for Use in the Diagnosis and Evaluation of Dry Eye Disease

By

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ABSTRACT

This dissertation presents methods for the evaluation of ocular surface protection during natural blink function. The evaluation of ocular surface protection is especially important in the diagnosis of dry eye and the evaluation of dry eye severity in clinical trials. Dry eye is a highly prevalent disease affecting vast numbers (between 11% and 22%) of an aging population. There is only one approved therapy with limited efficacy, which results in a huge unmet need. The reason so few drugs have reached approval is a lack of a recognized therapeutic pathway with reproducible endpoints. While the interplay between blink function and ocular surface protection has long been recognized, all currently used evaluation techniques have addressed blink function in isolation from tear film stability, the gold standard of which is Tear Film Break-Up Time (TFBUT).

In the first part of this research a manual technique of calculating ocular surface protection during natural blink function through the use of video analysis is developed and evaluated for it's ability to differentiate between dry eye and normal subjects, the results are compared with that of TFBUT. In the second part of this research the technique is improved in precision and automated through the use of video analysis algorithms. This software, called the OPI 2.0 System, is evaluated for accuracy and precision, and comparisons are made between the OPI 2.0 System and other currently recognized dry eye diagnostic techniques (e.g. TFBUT). In the third part of this research the OPI 2.0 System is deployed for use in the evaluation of subjects before, immediately after and 30 minutes after

exposure to a controlled adverse environment (CAE), once again the results are compared and contrasted against commonly used dry eye endpoints.

The results demonstrate that the evaluation of ocular surface protection using the OPI 2.0 System offers superior accuracy to the current standard, TFBUT.

DEDICATION

I would like to dedicate this doctoral dissertation to my father Dr. Mark Abelson,

and thank him for his guidance and encouragement.

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

The focus of this research is in the development of an accurate and precise
endpoint for the evaluation of ocular surface protection for use in the diagnosis
and evaluation of dry eye. Dry eye is a highly prevalent disease affecting vast
numbers (between 11% and 22%) (Brewitt & Sistani, 2001) of an aging
population. There is only one approved therapy with limited efficacy resulting in
a huge unmet need. The reason so few drugs have reached approval is a lack of a
recognized therapeutic pathway with accurate, reproducible endpoints. Compiling
the complexities in this area of development is a large array of trial designs as
well as constantly changing environmental factors. The value of a reliable
clinically meaningful endpoint can only be recognized in the context of a
therapeutic pathway in the approval process. A therapeutic pathway pertains
specifically to the efficacy of a drug and the way in which that drug acts on the
disease it is predicted to improve. It is this pathway that links how a disease is
diagnosed to the most efficacious treatment. Drugs are approved based upon two
main criteria, Safety and Efficacy.

As the audience of the papers contained in this dissertation are in the
medical field and therefore the clinical relevance of the methods developed herein
were of most interest rather than the engineering tools deployed to accomplish
this work, the style in which this dissertation reads is a bit different than that of

most engineering Dissertations; that being said the tool used to complete this work include Human factors engineering, DOE (Design of Experiments), computer science engineering, image analysis, Verification and Validation of systems, non-parametric modeling, generalized Linear models and six sigma methodologies.

This research was spawned out of identification Tear film Breakup Time (TFBUT), the gold standard measurement of tear film stability developed in 1973 and used worldwide by ophthalmologists and clinicians to evaluate dry eye severity, had unreasonably high variability. In analyzing the process it was determined that a full redesign of the process was necessary and that a higher degree of reliability and reproducibility could be achieved. Through the use of FMEA and the DMAIC process we redesigned, improved, optimized and stabilized the process. It was this engineering approach that made this research possible and through the deployment of the tools mentioned above successful.

Clinical development of an ophthalmic drug begins with submission of an Investigational New Drug (IND) application to the United States Food and Drug Administration (FDA).[1] This submission occurs after sufficient preclinical data determining the compound's reasonable safety and pharmacological activity is gathered, and marks the beginning of the FDA's involvement in the drug development process. The IND is required to include summaries of results of animal pharmacology and toxicology as well as any prior use in humans (typically

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[1] With the exception of ophthalmic formulations meeting the requirements of 21 CFR part 349 ("Ophthalmic Drug Products for Over-the-Counter Human Use").

pertains to foreign use); manufacturing information to ensure adequate production and batch consistency capabilities; and detailed protocols and investigator information for proposed clinical research. Following IND submission, the sponsor is required to wait 30 days prior to initiating clinical trials, during which time the FDA has the opportunity to review the IND for subject safety concerns.

The International Conference on Harmonisation (ICH) Guidance on General Considerations for Clinical Trials "discusses the recognized principles and practices in the conduct of clinical trials and the development strategy for new drug products"(1997) The clinical development pathway of a drug typically consists of three general phases, described as the following: Phase I as the initial administration of an investigational new drug in humans, Phase II being the exploration of therapeutic efficacy in patients, and Phase III as the demonstration or confirmation of the drug's therapeutic benefit. Phase IV studies are those performed subsequent to drug approval. In the design of clinical trials, the guidelines state that primary endpoints should be selected clinically relevant measurements. Secondary endpoints may or may not be related to the primary endpoint. Furthermore, the methodology involved in measuring these endpoints "should be validated and meet appropriate standards for accuracy, precision, reproducibility, reliability, and responsiveness (sensitivity to change over time)(FDA, 1997) ".

When the sponsor (i.e. pharmaceutical company, academic institution, etc.) believes that the results of these studies adequately demonstrate the drug's

safety and effectiveness, the next step is to submit a New Drug Application (NDA)(FDA, 2009). The NDA serves as an application to market the drug and includes all information available on the drug to-date as well as proposed labeling information in the form of a proposed package insert. The application must provide adequate information and analyses for FDA reviewers to review to determine: (1) whether the drug can be deemed safe and effective in its proposed uses, and whether the benefits outweigh any risks incurred by the drug; (2) whether the drug's proposed package insert is appropriate and what it should contain; and (3) whether the manufacturing methods and controls supply sufficiently preserve the drug's identity, strength, quality, and purity (FDA, 2009). No new drug can be legally marketed in the United States without FDA approval of an NDA, unless recognized as safe and effective for its intended use, as in the case of over-the-counter products described in a drug monograph(Lloyd, Harris, Wadhwa, & Chambers, 2008).

Within 60 days of the FDA receiving an NDA, the agency must determine filability of the application. In the case of incomplete or deficient applications, the FDA may take a refuse to file action. If the FDA determines that the package is contents are adequate for review, a filable action is taken. Once deemed filable, the agency has either 6 (priority review for therapies representing a significant therapeutic advance) or 10 (standard review) months to complete the regulatory decision (Lloyd, et al., 2008). The decision is based on the goal of establishing the safety and efficacy of the drug product for the indicated use. Although the

meaning of the phrase "safe and effective" can be subjective, the FDA language is

based on the Federal Food Drug and Cosmetic Act and explains that the

application must include "full reports of investigations which have been made to

show whether or not such drug is safe for use and whether such drug is effective

in use" (ICH, 2009; Schachat, Chambers, Liesegang, & Albert, 2003) . Advisory

committees (composed of FDA-appointed doctors, scientists, industry

representatives, etc) may be convened in order to provide public input on the drug

product, and provide nonbinding advice to the FDA regarding approval. At the

completion of NDA review, the FDA issues one of three regulatory actions:

approval, approvable, or not approvable; which denote marketability, requirement

of further information and potential additional clinical trial completion, and no

approval at this time (for any of a variety of reasons), respectively (Lloyd, et al.,

2008).

Efficacy Endpoints

The role of endpoints outlined in clinical trial protocols is to assess drug

effects (i.e. related to pharmacokinetics, pharmacodynamics, efficacy, and/or

safety) (FDA, 1997). There should be adequate evidence of the reliability and

validation of primary variables in regards to clinical relevance and treatment

benefit in the population studied (ICH, 2009). The methods used to measure both

subjective and objective endpoints, "should be validated and meet appropriate

standards for accuracy, precision, reproducibility, reliability, and responsiveness

(sensitivity to change over time)"(FDA, 1997).

Dry Eye: The unmet need for treatment

Estimates of dry eye prevalence vary with the populations studied and

parameters defining diagnosis, and reports range from roughly 11% to 22%

(Brewitt & Sistani, 2001) Normally, the human tear film is a complex solution of

various aqueous, lipid, and mucin components in a fragile balance, and protects

and nourishes the ocular surface. When the homeostasis is interrupted by any of a

multitude of factors, the tear film can become unstable and dry eye results.

Research over the years has unveiled risk factors that include use of systemic

medications with ocular drying effects (e.g. antihistamines, tricyclic

antidepressants, diuretics), systemic disease (e.g. autoimmune, rheumatic), and

altered innervation (e.g. damage to the fifth cranial nerve, metabolic deficiencies,

modified blinking patterns), but other risk factors such as cigarette smoking, acne,

and alcohol use are still under debate.(M. B. Abelson, Ousler, & Maffei, 2009)

("The epidemiology of dry eye disease: report of the Epidemiology Subcommittee

of the International Dry Eye WorkShop (2007)," 2007) Real-world challenges

such as prolonged visual tasking, contact lens wear, and exposure to windy, hot,

arid environments can destabilize the tear film or further endanger an already

compromised tear film as well.

The pathophysiologies encompassed under the blanket term of "dry eye" run the gamut of highly symptomatic patients who display no clinical signs associated with dry eye to patients demonstrating severe signs of dry eye, but who elicit minimal to no symptomatic complaints (G. W. Ousler, 3rd, Hagberg, Schindelar, Welch, & Abelson, 2008). Symptoms associated with dry eye include complaints of: burning, stinging, grittiness, discomfort, photophobia, blurred vision, etc. One dry eye patient may experience excessive tearing as a result of physiological compensatory attempts in response to insufficient lubrication, while another experiences constant symptoms of dryness and grittiness. Clinical signs include: conjunctival hyperemia (redness), ocular surface staining (cell damage), shortened tear film break-up time (TFBUT) (unstable tear film), decreased tear production, etc.

Once diagnosed, treatment is the next challenge. Over-the-counter (OTC) ocular lubricants (also known as "artificial tears" or "tear substitutes") are the mainstay in dry eye treatment today. The assorted formulations available include: single- or multi-dose packaging, preserved or unpreserved solutions, varied active and inactive ingredients, etc, and these formulations are marketed in accordance with the FDA monograph entitled, "Ophthalmic Drug Products for Over-the-Counter Human Use"(FDA, 1988). Studies have demonstrated prolonged TFBUT, enhanced ocular surface protection between blinks, and improved symptomatic conditions through the use of these drops, but the formulations

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typically provide only transient relief and apply mostly to mild-to-moderate cases of dry eye or as adjuvant to therapy in more severe cases.

A biodegradable polymeric ophthalmic insert designed for prolonged lubrication via insertion into the subconjunctival sac and subsequent dissolution by natural tears is also available by prescription, but can cause visual blurring and may present problems in patients with substantially decreased tear production (*Lacrisert [prescribing information]*, 2007). Only one eye drop, cyclosporine 0.05% (Restasis, Allergan) has been approved by the FDA for the treatment of dry eye, and even this therapy has a limited indication to, "increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca". The labeling also states that, "increased tear production was not seen in patients currently taking topical anti-inflammatory drugs or using punctal plugs" and that statistically significant increases in tear production was only observed in 15% of treated patients (*Restasis [prescribing information]*, 2009) Other modes of dry eye treatment include implantable punctal plugs (tears exit the eye through the punctum) for tear retention and corticosteroids and systemic tetracycline are used (for more severe cases), but the efficacy of the former is controversial and the latter two are off-label uses which introduce the risk of side effects (Pflugfelder, 2004).

Agents currently in development for the disease include those targeting the mucin components of the tear film (mucin secretagogues—agents that stimulate

mucin secretion and mucogenics—agents that synthesize mucin), anti-
inflammatory agents of varying mechanisms of action, and a combination
corticosteroid/iontophoretic device (see **Table 1.1**). Ideally, treatments will
eventually be tailored toward different pathophysiologies of dry eye. While FDA
approval presents a substantial challenge, the market situation illustrates a
considerable population of dry eye patients left underserved by available
treatments, and represents tremendous opportunity for clinical development
improvement and therapeutic advancement.

Table 1.1. *Agents in development for dry eye treatment*

Agent	Company	Class
Cyclosporine A	Novagali Pharma www.novagali.com	Anti-inflammatory
Dexamethasone phosphate via ocular iontophoresis	EyeGate Pharma www.eyegatepharma.com	Anti-inflammatory
Diquafosol tetrasodium	Inspire Pharmaceuticals www.inspirepharm.com	Mucin secretagogue
Doxycycline (nonantimicrobial)	Alacrity Biosciences www.alacritybio.com	Anti-inflammatory
Ecabet sodium	ISTA Pharmaceuticals www.istavision.com	Mucin secretagogue
MIM-D3	Mimetogen Pharmaceuticals www.mimetogen.com	Mucin secretagogue
Rebamipide	Otsuka Pharmaceuticals www.otsuka-global.com	Mucogenic/ Mucin secretagogue
RX-10045 (resolvin therapy)	Resolvyx Pharmaceuticals www.resolvyx.com	Anti-inflammatory
SAR 1118 (LFA-1 antagonist)	SARcode www.sarcode.com	Anti-inflammatory
Voclosporin	Lux Biosciences www.luxbio.com	Anti-inflammatory

Diagnostics and Clinical Models

Precise clinical models are integral to successful evaluation in drug
development; Still, many clinical trials consist purely of environmental exposure
components, which introduce variability between subjects (e.g. time spent at work

using a computer, time spent outdoors, relative humidity flux, etc). To control this variability, the Controlled Adverse Environment (CAE) model (Developed by Ora Inc.) was designed to exacerbate ocular signs and symptoms in a reproducible manner, but pertains to particular factors known to aggravate dry eye conditions rather than the allergic response. Lighting, airflow, temperature, humidity, and visual tasking parameters are precisely controlled in a chamber in order to provide a consistent challenge to the tear film and ocular surface that emulates real-world drying situations. The major advantage of the CAE model is that the environmental parameters are maintained in precise ranges, minimizing fluctuation (G. W. Ousler, Gomes, Welch, & Abelson, 2005). Typical CAE study designs include a screening visit to establish each individual's baseline response and ensure that this response is representative of the target treatment group, a confirmatory visit to ensure that the signs and symptoms observed at baseline are reproduced at a later time point, and various CAE re-challenges to treated eyes. The successful completion of numerous clinical studies utilizing the CAE model illustrates its applicability to the research and development in dry eye. (Crampton, et al., 2007b; Emory, Ousler III, & Abelson, 2003; Kellerman, et al., 2004; G. W. Ousler, 3rd, Abelson, Nally, Welch, & Casavant, 2002; G. W. Ousler, 3rd, Anderson, & Osborn, 2008; Ousler GW, Gomes PJ, Crampton HJ, & MB., 1999; G. W. I. I. I. Ousler, Canova, Nentwig, Welch, & Abelson, 2009; Ousler III, Haque, Weichselberger, Yannoulis, & Abelson, 2005; Ousler III, Welch, & Abelson, 2004; Pratt, Ousler III, Schindelar, Chapin, & Abelson, 2005)

Another clinical tool designed for dry eye research is the Ocular

Protection Index (OPI) (G. W. Ousler, 3rd, Hagberg, et al., 2008). The original

OPI is calculated as the quotient of TFBUT divided by inter-blink interval (IBI,

the average number of seconds between blinks). The TFBUT variable is assessed

by the clinician asking the patient to blink twice and then stare, and represents the

number of seconds between eye opening after the second blink (beginning of the

stare) and the appearance of the first expanding break in the tear film. The IBI

variable is typically calculated by capturing a fixed gaze blink rate (video-

recorded using a headset microcamera during completion of a standardized visual

task) and computing the average number of blinks per minute and subsequently

the average number of seconds between blinks. The OPI represents a binary

variable of average ocular surface protection: an OPI ≥ 1.0 denotes sufficient

ocular surface protection, while an OPI < 1.0 denotes insufficient ocular surface

protection. The latter situation may indicate a compromised ocular surface and the

need for tear film stabilization. The allure of this metric is two-fold: its simplicity

and, more importantly, its applicability across disease subpopulations. In essence,

because all manifestations of dry eye are characterized by tear film instability, the

OPI is a common denominator of all etiologies and pathophysiologies.

Technological advances have since been developed to more accurately

measure and represent the state of ocular surface protection and exposure supplied

by the tear film. (R. Abelson, et al., 2011) But unlike these other techniques the

methods developed in this research take advantage of real-time video capture and

subsequent analysis of blink rate and TFBUT simultaneously, thereby eliminating

the questions instilled by the separate capture of IBI and TFBUT in the original

metric. The analysis is performed using a computer program consisting of a

template matching algorithm and a specific threshold for indication of a blink in

the series of video frames (R. Abelson, et al., 2012). The enhanced metric also

allows for interpretation of partial blinks in addition to complete, and incorporates

real exposure calculation capabilities.

Meeting regulatory requirements

The technological advances made require compliance with several

regulatory guidelines. Because the OPI 2.0 system utilizes electronic capture and

analysis of data, it must meet the requirements of the Code of Federal

Regulation's "Electronic Records; Electronic Signatures". Key requirements

include system validation, ability to generate duplicate records for FDA

inspection, adequate protection of the records, use of secure and accurate audit

trails (via computer-generated time stamps when user creates, modifies, or deletes

records), electronic signatures, use of a series of checks (operational, authority,

device), and adequate training of system users (FDA).The document clearly states

that the procedures used in electronic records include system validation, "to

ensure accuracy, reliability, consistent intended performance, and the ability to

discern invalid or altered records". The revised OPI metric involves video capture

of patient data subsequent analyses, technician and doctor review, and storage of these data, all of which apply to the regulations for electronic records.

Revisiting the OPI metric, research identified shortcomings in the real-world applicability of the original OPI as well as potential opportunities for improved accuracy. In two identical clinical studies, results were found to be inconsistent for OPI while the results for other endpoints evaluated remained much more consistent. A review of the process through which Blink Rate and Tear Film Break Up Time were evaluated. Blink Rate was evaluated using a ISCAN[tm] (Burlington, MA) blink counter. The blink counter consists of a headset that fits over the head of the patient and a camera points at the eye under evaluation. As the blinks are recorded with the video camera a program in the background counts the blinks. If the camera is set up incorrectly or the patient is wearing eyeliner, the counter could have a difficult time accurately counting the blinks. As the program runs simultaneously to the evaluation and the videos are not saved, there is no method for any type of post-hoc evaluation of the accuracy of the video. Through manipulation of the system dump files with patient's blink data was created, and simultaneously another video camera was used to capture the actual blink. After gathering a few patients' data the blink rate was manually assessed from the recorded video and compared to the output from the blink counter.   Minimal analysis revealed that there was a substantial amount of error generated by the blink counter. Secondly, a review of the method for measuring Tear film Break Up Time was completed, which consists of an Ophthalmologist

watching the patient's eye through a slit lamp and assessing the time of break up using a stop watch. This is done once on each eye and a single sample is taken for each. Thereby, a patient could have two different measures of TFBUT with a single measure of blink rate. The Blink rate and TFBUT assessments are done at different time points and the OPI measurement calculated later. It was very apparent that this method for estimating if a patient's eye was protected was fraught with issues (shortcomings). In the list below find some of the major ones:

- Operator error

- Blink Rate Counter Machine error

- Sample size of TFBUT

- TFBUT evaluated at a different time than blink rate.

- Measurement error in both Blink Rate and TFBUT

There were two main opportunities identified. The first was that it would be of interest not only to evaluate the time of the cornea exposure (Calonge, 2001), but the actual area of exposure over time. The second opportunity was to evaluate Blink and Tear Film Break Up Time within a patient's normal blink pattern rather than Blink at one time point and TFBUT at another.

In the first paper, a manual technique to accurately measure the area of ocular protection during a normal blink pattern is presented. This technique while proven to be reasonably accurate and sufficient to meet the goal of differentiating between dry eye patients and normal patients, is very manually intensive and uses

estimation to calculate the area of corneal exposure over time. To make this a scalable technique that could be used as a viable endpoint on larger trials, the technique needed to become more efficient, less manual and less computationally intensive.

Therefore, in the second paper, an almost fully automated technique is presented using a video analysis program. This new methodology, outlined in the flow chart below (**Figure 1.1**), employs commonly used video analysis methods to detect pixel density differences between a baseline image of a fully protected tear film and subsequent images.

Figure 1.1. *Flow Chart of Video analysis method.*
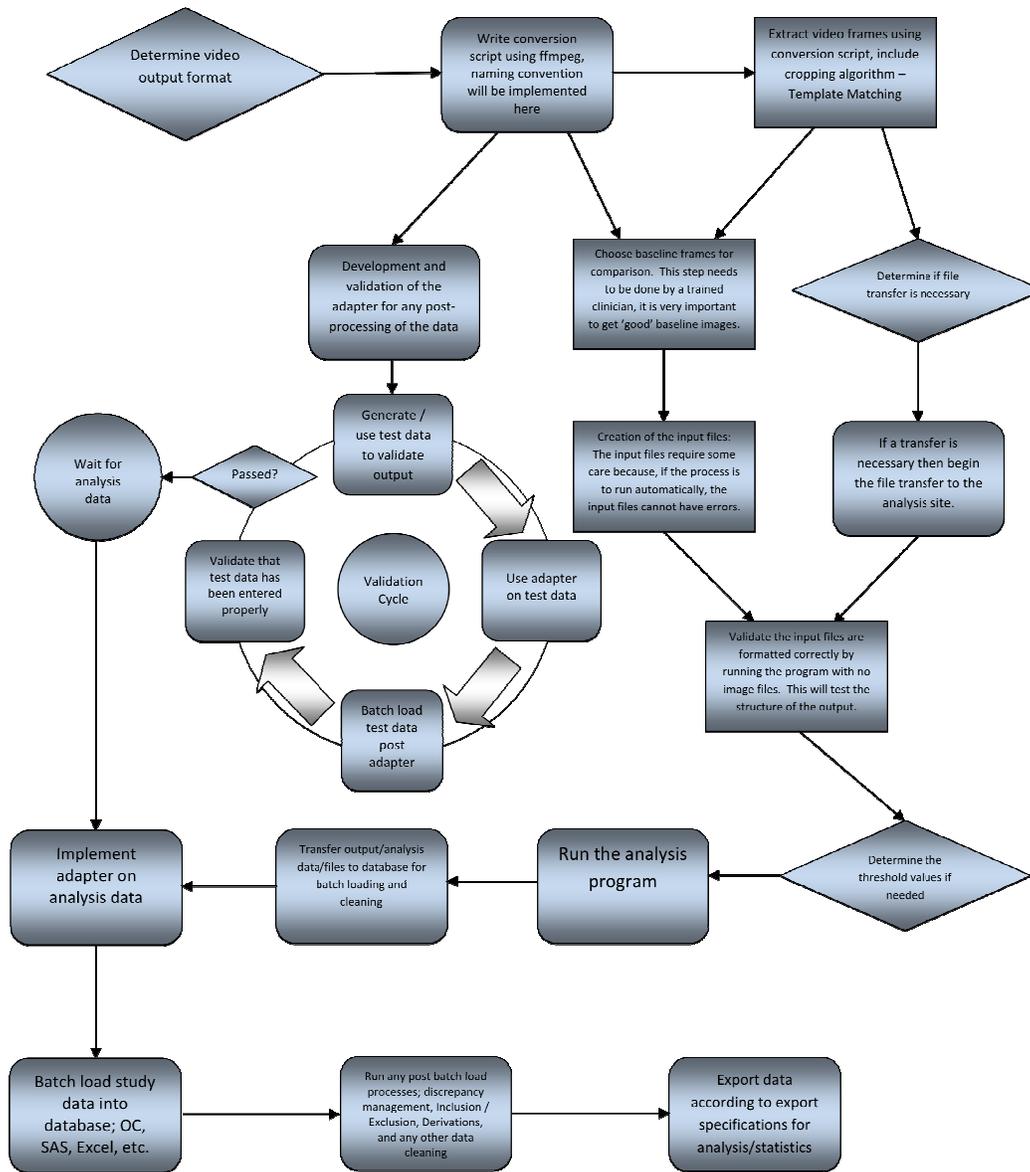
Through the use of these video analysis techniques, the percent of corneal

exposure in any given image can be estimated. As the video camera takes 15

frames per second and videos last approximately one minute, 900 data points are captured to reveal the performance of a given patient. This data, displayed graphically in **Figure 1.2** with Time on the x-axis and Percent of Corneal Exposure on the Y-axis, results in a very intuitive representation of the underlying pathophysiology of the eye at any point in time.

Figure 1.2. *Graph of Percent Exposed Cornea vs. Time.*
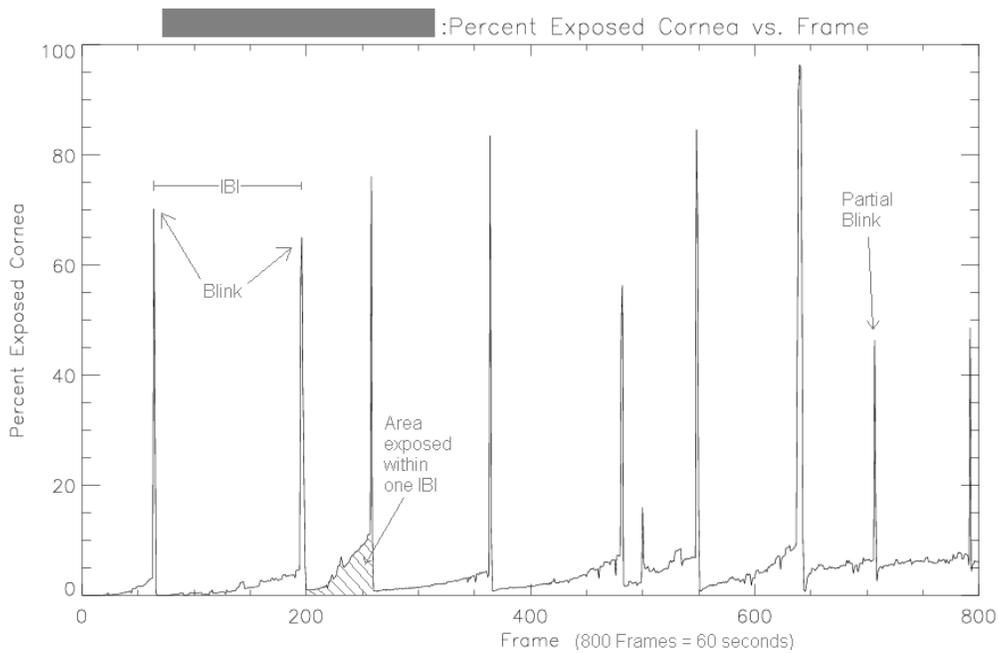
In the above graph the vertical lines represent blinks and the sloped lines in between the blinks demonstrate the percent of corneal exposure at that given time point. The increasing exposure over time until the patient blinks again is apparent.

At around 700 frames this particular patient has a partial blink that doesn't fully refresh the tear film.

After outlining and discussing the new analysis methodology of the video technique the second paper presents results showing the use of this tool in differentiating between dry eye and normal subjects. A detailed discussion of verification and validation techniques employed are also presented.

In the third paper the OPI 2.0 System is used to evaluate patients before, immediately after and 30 minutes after exposure in the CAE. The results demonstrate the additional information the MBA variable provides in understanding subject response. TFBUT is also evaluated at the same time points and demonstrates poor correlation to MBA, discomfort, staining and changes in fissure width. The data provided suggests that TFBUT lacks the precision to be a useful parameter in evaluating the signs of Dry Eye.

CHAPTER 2

MEASUREMENT OF OCULAR SURFACE PROTECTION UNDER

NATURAL BLINK CONDITIONS

Background

The ocular surface and its individual components make up the protective barrier

between the eye and the outside world. It is regularly challenged by the

environment (eg, low humidity, wind exposure, pollutants) as well as disease (eg,

autoimmune disease, neurologic disease).(G. W. Ousler, et al., 2005) In response

to these challenges, the ocular surface and its components are in a highly dynamic

state constantly adjusting to different environmental and biologic

conditions.(Rolando & Zierhut, 2001) Secretions from the main and accessory

lacrimal glands, meibomian glands, and conjunctival goblet cells provide the

aqueous, lipid, and mucin components, respectively, of the human tear

film.(Chao, Vergnes, Freeman, & Brown, 1980; Mishima & Maurice, 1961;

Nagyova & Tiffany, 1999; Nguyen, Beuerman, Meneray, & Maitchouk, 1998;

Rolando, Refojo, & Kenyon, 1985; Rolando & Zierhut, 2001) The tear film

serves three main functions: protection of ocular surface epithelial cells from

desiccation, nourishment of the epithelium, and optical refraction. Interruption of

the fragile homeostasis of the tear film via insufficiencies in either the quality or

quantity of its constituents can result in tear film instability and may lead to

surface damage. Such surface damage is often characteristic of the many pathophysiologies of dry eye disease.

The relationships between the time between successive blinks, or the interblink interval (IBI), and tear film breakup time (TFBUT), the time from the completion of a blink to the appearance of the first dry spot, or micelle on the cornea, define the integrity of the ocular surface.(M. B. Abelson, Ousler, Nally, Welch, & Krenzer, 2002; Holly, 1973; Lemp, 1973; Lemp, Goldberg, & Roddy, 1975; Smith, Nichols, & Baldwin, 2008) Accordingly, both IBI and TFBUT are meaningful variables to characterize in efforts to better understand dry eye. As a standard diagnostic test for over 40 years, TFBUT has been traditionally measured during a forced-stare following 2 forced, complete blinks by an observer with a stopwatch observing the fluorescein-stained ocular surface through a slit lamp.(M. B. Abelson, et al., 2002; G. W. Ousler, 3rd, Hagberg, et al., 2008)

The Ocular Protection Index (OPI) was developed to capture the nature of the interaction between blinking and TFBUT, and the OPI methodology has been used in numerous observational studies and clinical trials.(Crampton, et al., 2007a; D'Arienzo, Ousler III, & Schindelar, 2007; G. W. Ousler, Emory, Welch, & Abelson, 2002b; G. W. Ousler, Michaelson, & Christensen, 2007; Wilcox Hagberg, Ousler III, Casavant, Welch, & Abelson, 2005b) The OPI is calculated by dividing the TFBUT by the inter-blink interval (IBI).(G. W. Ousler, 3rd, Hagberg, et al., 2008) In an ideal state, tear film break up does not occur prior to

the next blink (i.e. TFBUT > IBI). Based on this assumption, if the OPI is < 1, a patient's cornea is considered at risk for exposure, resulting in the development or exacerbation of dry eye signs and symptoms, and if the OPI is $\geq$ 1, a patient's cornea is considered to be protected, presumably resulting in fewer dry eye signs and symptoms.(G. W. Ousler, 3rd, Hagberg, et al., 2008)

While the use of OPI provides context for determining the clinical relevance of TFBUT, our increased understanding of the complexities of blink physiology and tear film breakup suggests that the traditional methodology has a number of shortcomings:

(1) *Data collected at different times*: The TFBUT measurement and the IBI measurement are performed at different times. Blink rate is captured under normal blink conditions while the subject watches video, while TFBUT is measured separately.

(2) *Data collected under unnatural physiological conditions:* TFBUT is evaluated using the forced-stare technique, which is an unnatural physiological condition.

(3) *Potential confounding factors:* The forced stare may introduce complications such as reflex tearing and increased ocular discomfort. The manual measurement of TFBUT with a stopwatch introduces imprecision and variability. The use of a stopwatch innately introduces human error into the manual measurement of TFBUT as there is an inherent delay between the time the doctor can detect a break and the time the stopwatch is stopped.   The blink rate method used (video

capture headset and associated software) counts only complete blinks. The inclusion of other types of blinks in the evaluation should yield a more accurate depiction of the degree of protection at the corneal surface. In addition, the use of a single time provides no information on the area of corneal surface exposed, or rate of the change in the exposed area as a function of time during the IBI.

To address the shortcomings of the traditional (Forced-Stare, FS) methodology, this paper evaluates an alternative method for the evaluation of ocular surface protection under normal visual conditions. Briefly, the method involves retrospective analysis of video data of fluorescein-stained eyes taken through a slit lamp while the subject watches television. The retrospective analysis provides the area of tear film breakup for each IBI during the one-minute video. This technique is called Video Capture Manual Analysis (VCMA) and is described in more detail below. A study was performed and data are presented that compare the traditional (FS) and new (VCMA) methodologies. We demonstrate the ability of the new (VCMA) method to distinguish between normal and dry eye subjects and to identify post-treatment changes in dry eye subjects following the instillation of an artificial tear solution.

Methods

Measurement Techniques

Table 2.1 provides a list of definitions of variables analyzed.

Table 2.1. *Definitions of variables analyzed*

Measured Variables	Definition
TFBUT (breakup time)	Time in seconds from a blink until the first appearance of tear film break up
IBI (inter-blink interval) **traditional**	Time in seconds between complete blinks (>95% of the pupil covered)
IBI (inter-blink interval) **new**	Time in seconds between any blinks
BUA (breakup area)	Fraction of the cornea surface showing evidence of tear film breakup, as measured with the 17-zone corneal transect, at the end of the IBI. Units are % (percent of the corneal surface showing breakup)
Rate	Rate of increase in breakup area as a function of time during the time-exposed interval (see Figure 2). Units are % per second
Derived Variables	**Definition**
BUA/IBI	Breakup area divided by the inter-blink interval. Units are % per second

Traditional (FS) Method:

Primary-Gaze Blink Rate: Blink rate was measured using the IScan™ system (Burlington, MA) which consists of a headset (including a digital micro-camera and an infrared illuminator to track the diameter of the pupil) worn by the patient to non-invasively record blinks. Only complete blinks were counted, defined as > 95% of pupil coverage. During the blink rate evaluation, subjects were isolated

and were asked to watch a video image. The IBI was calculated by dividing the total number of complete blinks by the total time.

Forced-Stare TFBUT: Sodium fluorescein solution (5µl, 2% preservative-free) was instilled into the inferior conjunctival cul-de-sac of each eye and the subject was asked to blink several times to mix the fluorescein with their tear film. The subject was then asked to blink twice (squeeze-blinks) and then stare without blinking for as long as possible. The examiner monitored the integrity of the tear film through a slit-lamp biomicroscope with an 8 mm scanning beam (using an excitation blue filter and a barrier Wratten #12 yellow filter), and measured the time from eye opening to the first appearance of micelles with a stopwatch. The eyes were evaluated sequentially (OD, OS). Two measurements were taken and averaged unless the two measurements were both less than 10 seconds and differed by more than 2 seconds, in which case a third measurement was taken and the two closest of the three were averaged.

New (VCMA) Method:

Video of Fluorescein-Stained Eyes: Sodium fluorescein was instilled as described above. While the subject performed a standard visual task (watching a documentary on television from a five foot viewing distance), the eye was recorded using a digital video camera (EYECAP IM 900 camera system) at 8x magnification through a slit-lamp biomicroscope using an excitation blue filter

and a barrier Wratten #12 yellow filter. A minimum of one minute of continuous

data was recorded for each eye with roughly a thirty second pause between

recordings of the two eyes. The eyes were recorded from right (OD) to left (OS).

Retrospective Manual Analysis: A retrospective analysis of the data from each

eye was performed to generate TFBUT, IBI, and BUA over the 1 minute period.

A panel of examiners evaluated the integrity of the tear film and determined IBI

and TFBUT by manually stopping the video to note and confirm the time stamp,

and record the time of each blink and the first appearance of a micelle within each

IBI.

Videos were analyzed for BUA using a corneal transect comprising 17

sections overlaying the cornea (regions A – Q in **Figure 2.1**). The presence or

absence of breakup was graded for each applicable region (transect regions were

deemed "not applicable" if they enclosed non-corneal anatomy alone). For

example, in **Figure 2.1**, regions M, J, and I show areas of breakup. The BUA (%

area exposed) in **Figure 2.1** would be calculated as the areas of regions M, J, and

I, divided by the total of areas A through Q. If a portion of the region had

breakup, the whole area was deemed to have breakup and was included in the

calculation of BUA. The total number of regions ranged from 15 to 17 depending

on the position of the lids (e.g. if the upper lid covered the top two regions, only

15 areas were included).

Figure 2.1. *Corneal transect grid used to score corneal regions*
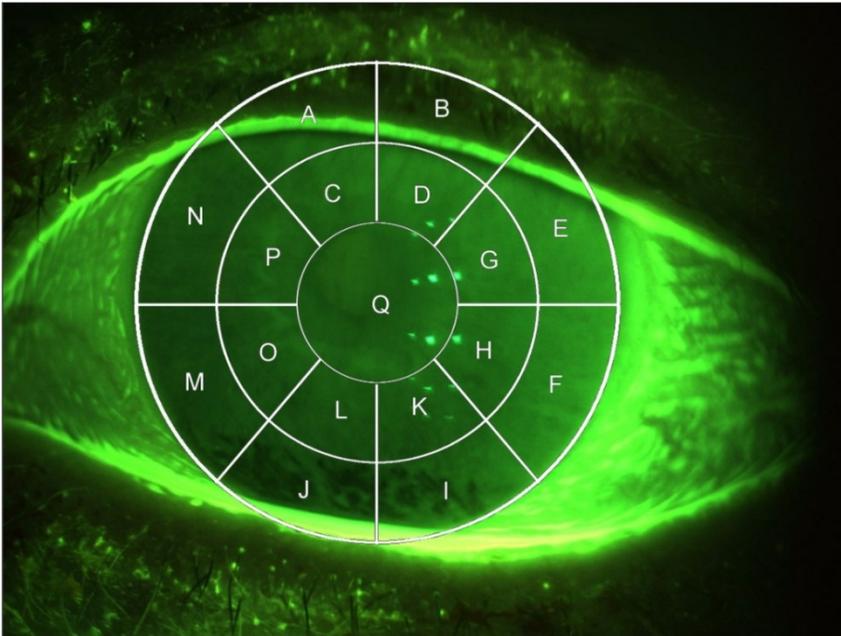
Figure 2.2 shows an example schematic diagram of the percent of corneal

exposure vs time during a single IBI used to calculate BUA. In this example, the

IBI is assumed to follow a partial blink, potentially leaving tear film defects, with

the consequence that the initial percent of area exposed is non-zero as depicted by

the diagonal cross hatch area in **Figure 2.2**. At some point during the IBI, the tear

film breakup area begins to increase, and this defines the TFBUT. The rate of

increase between TFBUT and end of the IBI is represented by the triangular area

at the right of **Figure 2.2**. The manual analysis of the video data provided

measurements of the percent cornea exposed at time 0 (immediately following a

blink), at the point of increasing break up area (TFBUT), and of the maximum

level of tear film breakup at the end of the IBI. Sequences of these three

measurements form sequences of schematic diagrams such as that shown in

Figure 2.2. From each diagram, BUA was calculated and these were averaged to

give mean values for the 1 minute observation period. The units of BUA are (%

cornea exposed)(sec). The IBI minus the TFBUT represents the "time-exposed

interval" (TEI), which can be expressed as a fraction of the IBI. The steepness of

the increase in BUA after the TFBUT allows analysis of tear film breakup rates.

Figure 2.2. *Schematic diagram of % corneal area exposed vs time during a*

single IBI
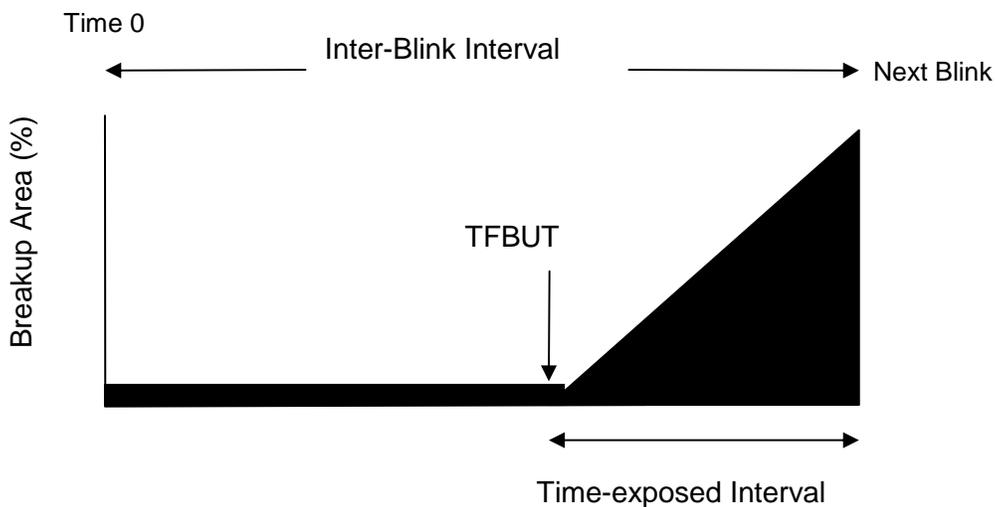
Study Design

This single-center, single visit, proof-of-concept pilot study was conducted

according to a protocol approved by an external Independent Review Board.

Written informed consent was obtained prior to study procedures. Patient-

reported and investigator-observed adverse events were captured and monitored for the duration of the study.

This study evaluated both eyes of 10 normal and 17 dry eye subjects. Enrolled subjects were at least 18 years of age, demonstrated a corrected visual acuity of +0.6 logMAR or better in each eye (Early Treatment of Diabetic Retinopathy Study), and were able and willing to avoid ophthalmic medications for 2 hours prior to each study visit. Subjects were excluded from the study if they wore contact lenses; had any ocular inflammation, ocular infections, active ocular inflammation or preauricular lymphadenopathy; had any significant illness that could be expected to interfere with the trial parameters; had any known allergy or sensitivity to the test article or its components; had a condition that may have put the subject at significant risk, may have confounded the study results or may interfered significantly with the subjects participation in the study; or had taken any systemic medications known to cause ocular drying on an unstable dose within 14 days prior to the visit. Smokers were not excluded from the study. Dry eye subjects were selected based on reported use of artificial tears (no minimum use required) and were able and willing to avoid ophthalmic medications for 2 hours prior to the visit.

Dry eye subjects were measured by both the new (VCMA) and traditional (FS) methods, while normal subjects were measured by the new (VCMA) method only. Subjects underwent medical and medication history collection, subject-graded ocular symptom grading, visual acuity, and slit-lamp biomicroscopy.

After a five-minute resting period, conjunctival redness based on the Ora scale (0 [none] to 4 [severe]), and corneal sensitivity were measured. After a second five-minute resting period, primary-gaze blink rate was measured (traditional method IBI). After a third five-minute resting period, evaluations for the new (VCMA) method comprised tear film breakup time (TFBUT), interblink interval (IBI), and breakup area (BUA) based on the 1 minute video capture. Evaluations for the traditional (FS) method comprised the previously obtained primary-gaze blink rate and forced-stare TFBUT.

Following these evaluations, dry eye subjects were treated bilaterally with Refresh Liquigel. One to two drops per eye (OD, OS) were instilled by a technician and confirmed by a second technician. Subjects then repeated the aforementioned evaluations 10 (± 1) minutes after artificial tear instillation. For the purpose of this paper, the treatment effect was assessed by the VCMA method only.

In summary, the three paradigms relevant to this paper were as follows. First, traditional and new methods were used to measure the same set of 34 dry eyes prior to treatment. Second, the new method was used to measure 20 normal eyes and 34 dry eyes prior to treatment. Third, the new method was used to measure for the same set of 34 dry eyes before, and 10 minutes after, treatment with artificial tears.

Statistical Analysis

For each eye, derived variables were obtained as averages over the 1 minute video period. These outcomes were used to compare groups using a gamma multiplicative model estimated by generalized estimating equation methods (See Appendix C). These models provided estimates for group means, ratios of means, 95% confidence intervals, and P-values for a test of the equality of means. All models were fit using the genmod procedure of SAS version 9.2.("SAS Institute Inc. 2009. SAS OnlineDoc® 9.2. Cary, NC: SAS Institute Inc,")

The comparison between dry eye (34 eyes) and normals (20 eyes) was based on 54 eyes in two independent groups. The age-adjusted version of this model was based on a two-factor ANCOVA structure with interaction, with groups compared at 47 years, the mean age of the sample. A comparison of mean ages for dry eye and normal subjects was based on a t-test.

Comparisons between traditional (FS) and new (VCMA) methodologies (prior to treatment), as well as between pre-treatment and post-treatment means, were based on the same sample of 34 dry eyes. The correlation between groups was accommodated for via a sandwich variance estimator based on a working independence correlation structure.

Results

The mean ages for the normal (N=10) and dry eye (N=17) subjects were 60.8 and

24.0 years, respectively. Five normal subjects and 14 dry eye subjects were

female.

Comparison of Traditional (FS) and New (VCMA) Methods

Interblink Interval: **Table 2.2** shows the IBI data from the traditional (FS) and

the new (VCMA) methods the 17 dry eye subjects. The mean IBIs for the

traditional (FS) and new (VCMA) methods were 4.04 and 5.51 seconds,

respectively, for a ratio of 1.36 (P = 0.043).

Table 2.2. *Comparison of new (VCMA) and traditional (FS) methods in dry eye*

subjects

Variable	New[a] (N = 34)	Traditional (N = 34)	Difference (95% CI)	Ratio (95% CI)	P-value[b]
Time					
IBI (sec)	5.51	4.04	1.47	1.36 (1.01, 1.84)	0.043
TFBUT (sec)	3.98	5.82	-1.84	0.68 (0.54, 0.87)	0.002
TFBUT-truncated (sec)[c]	3.98	3.37[1]	0.61	1.18 (0.84, 1.67)	0.348

[a] N= number of eyes
[b] P-values based on correlated gamma multiplicative model
[c] TFBUT > IBI set equal to IBI for the traditional method (forced-stare)

Figure 2.3 shows histograms for both methods and a scatter plot for individual

data points.

Figure 2.3. *IBI observations for new (VCMA) and traditional (FS) methods for*

34 dry eye subjects. Figure 3a shows observed (yellow) and modeled (blue

lognormal) histogram, while figure 3b shows a scatter plot of the new vs

traditional observations relative to a 45 degree reference line. Sample means

were 5.5 for VCMA and 4.0 for FS.

(a)   (b)
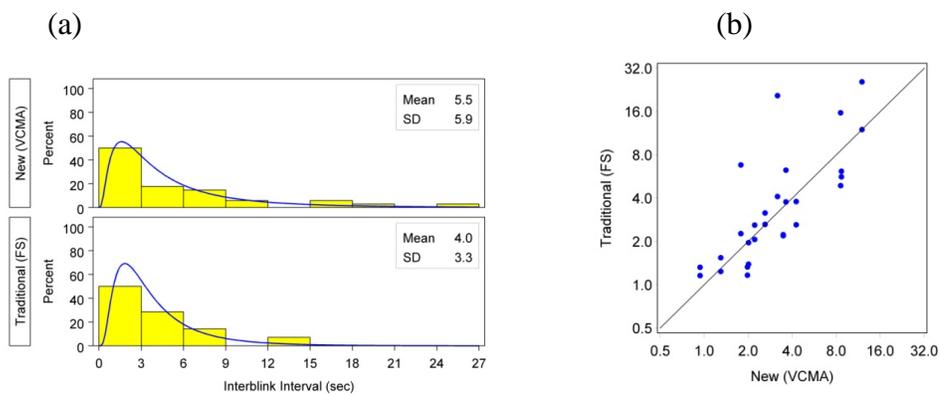
Tear Film Breakup Time: The mean TFBUTs for the traditional (FS) and the new

(VCMA) methods were 5.82 and 3.98 seconds, respectively, for a ratio of 0.68 (P

= 0.002), reflecting the very different methods used to measure these values. To

provide a more meaningful comparison, TFBUTs for the traditional (FS) method

were truncated to the corresponding IBI when no TFBUT was observed. This

approach gave similar means with a ratio of 1.18 (P = 0.348). **Figure 2.4** shows

histograms of both methods and scatter plots for individual data points. **Figure**

2.5 shows the corresponding plots using the truncated TFBUT values for the traditional (FS) method.

Figure 2.4. *TFBUT observations for new (VCMA) and traditional (FS) methods for 34 dry eye subjects.* Figure 4a shows observed (yellow) and modeled (green lognormal) histogram, while figure 4b shows a scatter plot of the new vs traditional observations relative to a 45 degree reference line. Sample means were 4.0 for VCMA and 5.8 for FS.
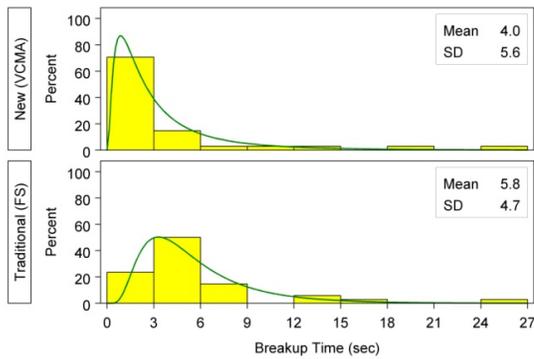
(a)   (b)
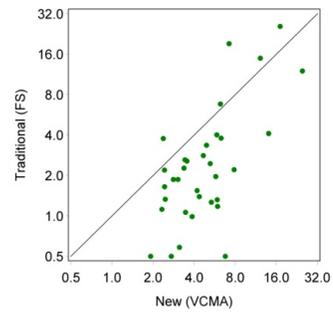
Figure 2.5. *Truncated TFBUT observations for new (VCMA) and traditional (FS)*

methods for 34 dry eye subjects. Figure 5a shows observed (yellow) and modeled

(brown lognormal) histogram, while figure 5b shows a scatter plot of the new vs

traditional observations relative to a 45 degree reference line. Sample means

were 4.0 for VCMA and 3.4 for FS.

(a)   (b)
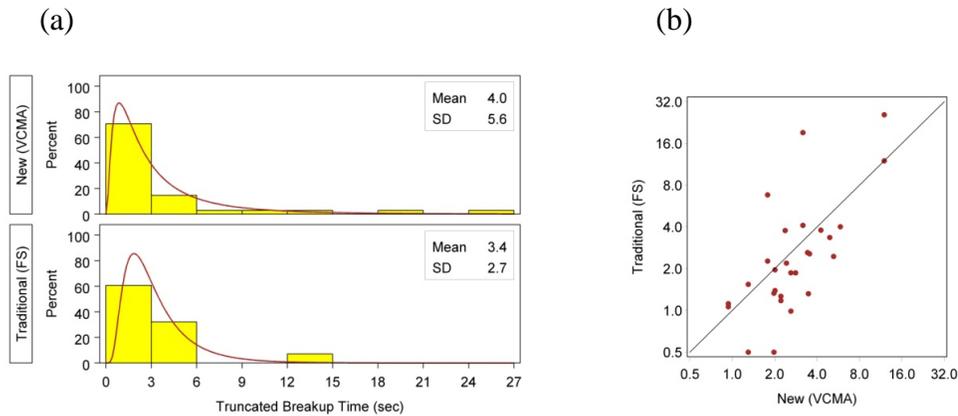
Comparison of Dry Eye and Normal Subjects

Table 2.3 summarizes group comparisons for dry eye and normal subjects for all

observed variables (IBI, TFBUT, BUA) and derived variables (BUA/IBI, Rate).

Mean IBIs for the dry eye and normal groups were 5.51 and 6.82, respectively, for

a ratio of 0.81 (P = 0.315). Mean TFBUTs were 3.98 and 5.39, respectively, for a

ratio of 0.74 (P = 0.200). Mean BUAs were 10.61 and 3.42, respectively, for a

ratio of 3.10 (P = 0.004).

Table 2.3. *Comparison of dry eye and normal subjects*

Variable	Dry Eye Subjects (N = 34)[a]	Normal Subjects (N = 20)	Difference (95% CI)	Ratio (95% CI)	P-value[e]
Time					
IBI (sec)	5.51	6.82	-1.31	0.81 (0.53, 1.22)	0.315
TFBUT[b] (sec)	3.98	5.39	-1.41	0.74 (0.46, 1.17)	0.200
Area					
BUA	10.61	3.42	7.19	3.10 (1.45, 6.65)	0.004
BUA/IBI[c]	3.70	0.45	3.25	8.22 (3.77, 17.91)	< 0.001
Other					
Rate[d]	7.67	2.37	5.30	3.24 (1.57, 6.66)	0.001

[a] N= number of eyes
[b] Video-capture-derived TFBUT: TFBUT > IBI set equal to IBI
[c] BUA/IBI (%) = BUA (% of cornea exposed x sec) divided by IBI (sec)
[d] Rate (%/sec) = rate of increase in BUA (% cornea exposed) / sec
[e] P-values based on gamma multiplicative model (Sample output can be seen in APPENDIX D)

Groups were compared with respect to two new derived outcomes: BUA/IBI, and rate of increase in BUA. BUA/IBI (in units of % corneal surface/sec) represents the fraction of corneal surface at risk or exposed. For the dry eye and normal groups, BUA/IBI means were 3.70 and 0.45, respectively, for a ratio of 8.22 (P < 0.001). **Figure 2.6** shows the relationship between BUA and IBI. Values for normal subjects clustered in the center of the IBI axis, while dry eye subjects were distributed across a wider range of IBI values and displayed elevated BUA values. For the dry eye and normal groups, the mean rate of increase in BUA was 7.67 and 2.37, respectively, for a ratio of 3.24 (P = 0.001).

Figure 2.6. *Scatter plot of Breakup Area(BUA) vs. Inter-blink Interval (IBI).* The parameter BUA/IBI represents the fraction of the corneal surface that is at risk (exposed); the units are % corneal surface/sec. The BUA/IBI data are represented for normal subjects (circles) and dry eye subjects (crosses).
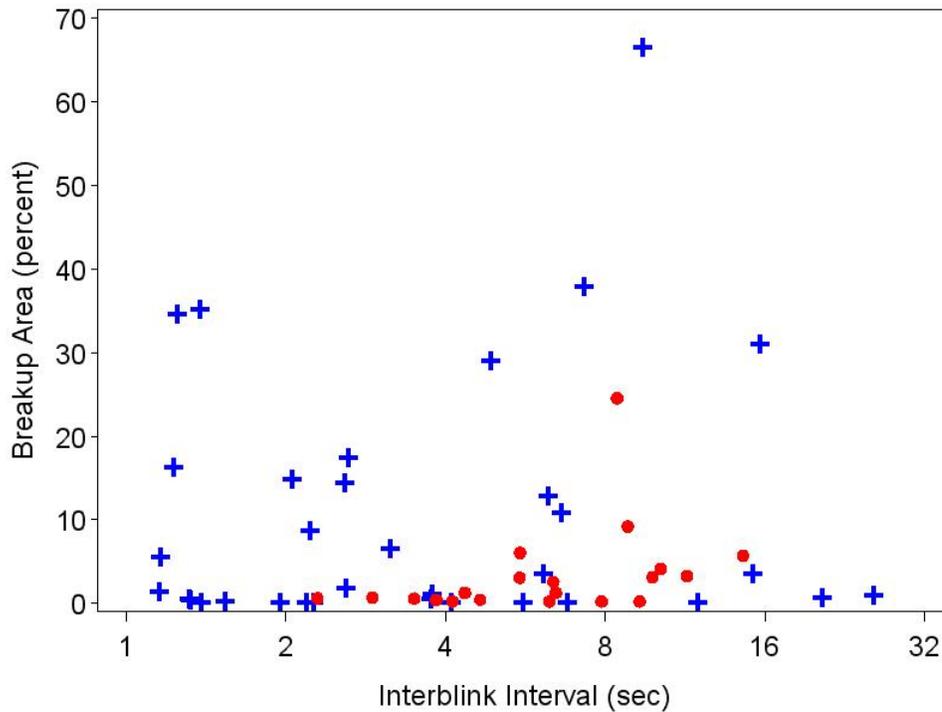
The above comparisons were based on unadjusted comparisons and thus may be influenced by other differences between the two groups. Indeed, groups did differ with respect to mean age (normal = 24 and dry eye = 60.8, P< 0.001), and for this reason the data were fit using an age adjusted model. The age adjusted results were qualitatively similar (**Table 2.4**).

Table 2.4. *Comparison of dry eye and normal subjects adjusted for age*

Variable	Dry Eye (N = 34)[a]	Normal (N = 20)	Difference (95% CI)	Ratio (95% CI)	P-value[e]
Time					
IBI (sec)	7.14	3.26	3.88	2.19 (0.39, 12.17)	0.371
TFBUT[b] (sec)	5.57	3.87	1.70	1.44 (0.22, 9.27)	0.701
Area					
BUA	4.07	0.05	4.02	74.6 (4.3, 1303)	0.003
BUA/IBI[c]	1.07	0.02	1.05	59.6 (3.1, 1132)	0.007
Other					
Rate[d]	3.85	0.01	3.84	364.6 (20.5, 6488)	< 0.001

[a]N= number of eyes
[b]bVideo-capture-derived TFBUT: TFBUT > IBI set equal to IBI
[c]BUA/IBI (%) = BUA (% of cornea exposed x sec) divided by IBI (sec)
[d] Rate (%/sec) = rate of increase in BUA (% cornea exposed) / sec
[e] P-values based on age-adjusted gamma multiplicative model

Detection of Treatment Effect

Table 2.5 summarizes group comparisons for dry eye subjects pre- and post-treatment with artificial tears for all observed variables (IBI, TFBUT, BUA) and derived variables (BUA/IBI, Rate).   Mean IBIs post- and pre-treatment were 7.70 and 5.5, respectively, for a ratio of 1.40 (P = 1.118). Corresponding means for TFBUT were 6.50 and 3.98 (ratio = 0.74, P = 0.034), and for BUAs were 6.75 and 10.61 (ratio = 0.64, P = 0.091). In the case of the derived variables, for the post- and pre-treatment groups, BUA/IBI means were 2.16 and 3.70 (ratio = 0.59, P = 0.001), and for BUAs were 6.75 and 10.61 (ratio = 0.64, P = 0.091).

Corresponding mean rates of increase in BUA were 15.39 and 15.30 (ratio = 1.01,

P = 0.985).

Table 2.5. *Comparison of treatment effect in dry eye subjects*

Variable	Post Instillation (N = 34)[a]	Pre Instillation (N = 34)	Difference (95% CI)	Ratio (95% CI)	P-value[e]
Time					
IBI (sec)	7.70	5.51	2.19	1.40 (0.92, 2.12)	0.118
TFBUT[b] (sec)	6.50	3.98	2.53	0.74 (1.04, 2.57)	0.034
Area					
BUA	6.75	10.61	-3.87	0.64 (0.38, 1.07)	0.091
BUA/IBI[c]	2.16	3.70	-1.53	0.59 (0.42, 0.81)	0.001
Other					
Rate[d]	15.39	15.30	0.09	1.01 (0.56, 1.82)	0.985

[a]N= number of eyes
[a][b]Video-capture-derived TFBUT: TFBUT > IBI set equal to IBI
[c]BUA/IBI (%) = BUA (% of cornea exposed x sec) divided by IBI (sec)
[d] Rate (%/sec) = rate of increase in BUA (% cornea exposed) / sec
[e] P-values based on correlated gamma multiplicative model

Figure 2.7 shows BUA vs IBI for the dry eye subjects pre- and post-instillation of

artificial tears. Even though the mean values for BUA and IBI were different,

there is no obvious separation of the groups.

Figure 2.7. *BUA (% corneal surface) versus IBI (seconds) for 34 dry eyes before (blue crosses) and after (green stars) instillation of artificial tears.*
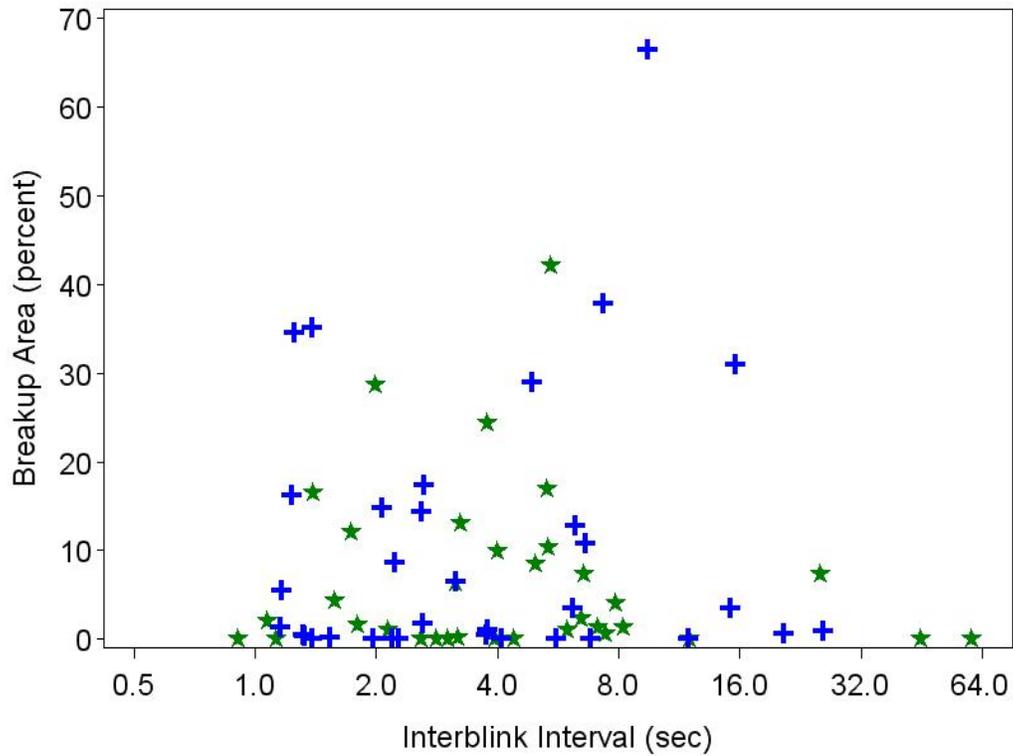
Discussion

This paper introduces a new method for evaluating ocular surface protection under normal visual conditions and, as such, is more clinically relevant than the traditional Forced-Stare method. A key feature of the new VCMA method is that it allows for the simultaneous capture of TFBUT, IBI, and BUA while the subject is blinking normally.   While forced-stare TFBUT certainly identifies abnormalities in the tear film of dry eye subjects relative to normal subjects (as evidenced by over 30 years of reports(M. B. Abelson, et al., 2002; Holly, 1973;

Lemp, 1973; Lemp, et al., 1975; Smith, et al., 2008)), the new VCMA method affords this comparison in the natural setting.

One objective of this study was to compare the traditional (FS) and the new (VCMA) methods. To best understand the advantages of the VCMA method, it is of interest to compare the methods in terms of the traditional (FS) variables: IBI and TFBUT. In the VCMA method, IBI and TFBUT were recorded under natural conditions. In contrast, in the traditional (FS) method, TFBUT is recorded under forced-stare conditions and IBI under natural blink conditions. Despite the fact that IBI was recorded under natural conditions for both methods, the significant difference observed in this study between the IBI values generated by the two methods could reflect the fact that the blink counter equipment used in the FS method only counted complete blinks, whereas the VCMA method counted all blinks. The two methodologies are fundamentally different in the measurement of TFBUT. In the VCMA method, TFBUT is captured in a natural state while in the FS method, it is not. As a consequence, comparisons of TFBUT between the two methods require that the TFBUT from the traditional (FS) method be truncated at a value equal to the IBI (because in the new VCMA method, TFBUT cannot exceed the IBI). Analysis using the truncated data allows for both methods to be compared in a meaningful way.

A second objective of this study was to compare dry eye and normal subjects. In this study, as expected, dry eye subjects had lower IBIs and TFBUTs than normal subjects, although neither difference was statistically significantly.

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However, BUA, BUA/IBI, and the rate of increase of BUA were significantly different between the dry eye and normal subjects, indicating the diagnostic utility of these new variables. It appears that some dry eye subjects compensate for tear film instability and ocular surface discomfort by blinking more rapidly, thus avoiding elevated levels of BUA. The value of the derived variables in the VCMA method, in particular BUA/IBI, is the ability to identify both compensating and non-compensating subjects. We note that differences in BUA and rate between dry eye subjects and normal subjects have been reported elsewhere, but these authors collected the TFBUT and BUA data under forced-stare conditions. (Begley, et al., 2006; Liu, et al., 2006) While we acknowledge that the age difference between the groups may be a potential limitation of this study, an age adjusted analysis of the data provided qualitatively similar results.

The final objective of this study was to compare the effect of treatment with artificial tears in dry eye subjects. The area variables (BUA, BUA/IBI) were both able to detect a treatment effect. The analysis made possible by the VCMA methodology indicated that the treatment with artificial tears increased TFBUT but had no effect on rate of increase in BUA.

One potential limitation of this study involves the corneal transect grid. The corneal grid was chosen as more precise interpretation of the NEI scale for inclusion of more detail and to add specificity, although according to the grid method, any breakup in a region is deemed breakup in the entire region. This

may reduce precision and overestimate breakup, however the use of ratios of breakup means in the analysis should minimize any bias.

In summary, there is clinically relevant value in an analysis based on tear film stability measured in the context of a natural blink pattern.   While the traditionally used variables of IBI and TFBUT are useful, the data presented in this paper suggest that BUA is an important additional variable. Furthermore, BUA/IBI illustrates the potential of combining BUA with traditional variables. The manual data analysis used in this study was time consuming but provided the proof of principle. Studies are underway to automate the data collection and analysis process.

CHAPTER 3

VALIDATION AND VERIFICATION OF THE OPI 2.0 SYSTEM

Background

Reduced tear film stability is a key driving factor in the development of dry eye.

The measurement of tear film breakup time (TFBUT) using fluorescein with

forced-stare is a well established clinical metric for evaluating the health of the

pre-corneal tear film.(M. B. Abelson, et al., 2002; Holly, 1973; Lemp, 1973; G.

W. Ousler, 3rd, Hagberg, et al., 2008) More recently, the development of the

ocular protection index (OPI) was an important step in evaluating the interaction

between blinking and TFBUT. This tool has been used in numerous

observational studies and clinical trials and has been widely adopted by

clinicians.(Crampton, et al., 2007b; D'Arienzo, et al., 2007; G. W. Ousler, 3rd,

Hagberg, et al., 2008; G. W. Ousler, Emory, Welch, & Abelson, 2002a; G. W.

Ousler, et al., 2007; Rolando, Autori, Badino, & Barabino, 2009; Simmons &

Vehige, 2007; Torkildsen, Ousler, & Gomes, 2008; Wilcox Hagberg, Ousler III,

Casavant, Welch, & Abelson, 2005a)   However, our increased understanding of

the complexities of blink physiology and tear film breakup suggests that this

methodology has the potential to be improved upon. First, TFBUT and interblink

interval (IBI) measurements are performed at different times. Second, TFBUT is

evaluated using the forced-stare technique, which is not representative of the

physiological action of an unaltered blink pattern. Third, this methodology

provides no information on what occurs on the ocular surface between actual tear film breakup and the next blink, which is the point of corneal affliction.

In order to address these shortcomings, the OPI 2.0 System was developed to evaluate ocular surface protection under normal visual conditions. The approach yields a real-time measurement of percent cornea exposed (tear film breakup area or BA) for each IBI during a one minute video. The system also provides a simultaneous measurement of TFBUT and IBI. Utilizing this method, the mean breakup area (MBA) and the OPI 2.0, mean breakup area/interblink interval (MBA/IBI), are calculated and analyzed. Initially, a method of retrospective manual analysis of fluorescein staining video data was utilized with the OPI 2.0 System.(R. Abelson, et al., 2011) In this method, which we refer to as video capture with manual analysis (VCMA), a panel of examiners evaluated the integrity of the tear film and determined IBI and TFBUT by manually stopping the video to note and confirm the time stamp, and record the time of each blink and the first appearance of a micelle within each IBI. This method of BA evaluation utilized a sectoral transect of the corneal surface. Grading was made based on a binary evaluation of breakup within each region. A given region was counted as fully broken if any breakup was observed in that area regardless of the actual extent of exposure. Results utilizing the VCMA method demonstrated successful differentiation between normal and dry eye subjects; however, this methodology required numerous technician hours to manually grade the area of

corneal coverage and did not reach the desired level of precision.(R. Abelson, et al., 2011)

To improve the efficiency of the analysis, a complex set of algorithms were developed in order to automate the analysis of video footage collected. The processing of a video consists of two stages. The first is an image segmentation stage during which the corneal image is extracted from the background of the video frame using a template matching algorithm. The second stage consists of measurement of exposed area from the image sequence. The areas of exposure are summed pixel by pixel and divided by the mean corneal area over the entire video. This is to account for small variations in palpebral fissure width, and the calculation yields the average percent area of corneal exposure as a function of time.

The development of the software analysis had three goals: to calculate more precise values for the percent of corneal area exposed by way of computerized image analysis; to decrease human error (i.e. error introduced by the use of a stopwatch in the technician's calculation as there is an inherent delay between the time the doctor can detect a break and the time the stopwatch is stopped); and to increase the speed of analysis. Previous work on tear film breakup area has been conducted but it is uncertain how much validation has been completed on the procedures used.(Begley, et al., 2006; Harrison, et al., 2008; Jansen, Begley, Himebaugh, & Port, 2010) Advances in technology have prompted the use of video images to determine BA. While some techniques

measure BA from the last video frame before the IBI, the OPI 2.0 System is

designed to measure MBA, which is an average of the percent of the cornea

exposed over the entire video (**Figure 3.1**). The goal of adding software analysis

to the OPI 2.0 System was to accurately measure the amount of MBA on the

cornea and provide an efficient, clinically relevant measurement of the

pathophysiology of the ocular surface.

Figure 3.1. *Demonstrates data for one patient over 60 seconds of video.* MBA is

calculated as an average amount of corneal surface exposure over the entire video.
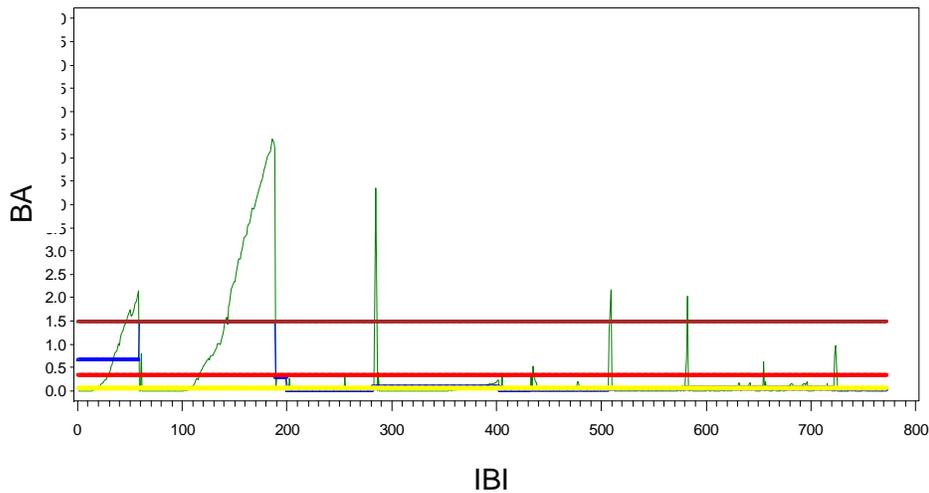
Methods:

Verification

To calibrate the software analysis and demonstrate that it can correctly identify

the area of exposure, a set of artificially constructed images were created to mimic

the visual properties of images captured during an actual clinical session using

fluorescein staining videography. The relevant parameters included density,

breakup dispersion, and image brightness. Breakup density represented the extent

of tear film breakup as a percentage of the corneal surface area. Breakup

dispersion represented the degree to which the exposed areas are distributed over

the corneal surface, ie the number of individual isolated regions of exposed

cornea. Image brightness represented the pixel intensity level of the green channel

of the image.

In addition to the eight images created to bracket the range of values of the

three parameters (designated HHL for high dispersion, high density and low

brightness, etc), a middle image was created at the mean parameter values to

create a total of nine images. To measure the effectiveness of the software, an

image was output with the areas of detected simulated tear film breakup shown in

red. For the purposes of this verification procedure, the artificially constructed

images created to mimic the visual properties of images captured during an actual

clinical session using fluorescein staining videography will be referred to as the

"artificial" images. The software analysis output of the image with the areas of

detected simulated tear film breakup will be referred to as the "detected" images.

There can be two types of incorrect detections of breakup area with regard to any discrepancies seen between the number of pixels detected in real images and the detected images: false negatives and false positives. A false negative detection is seen when breakup in the real image is not observed by the software analysis in the detected image. A false positive is seen when the software analysis detects breakup in the detected image that is not considered breakup in the real image.

Validation

The second stage involved using actual video images collected during the clinical validation process. The image properties were selected to correspond to the range of image values similar to the artificial images. After the selection, the images were graded manually by an expert grader and areas of exposed cornea were marked using image editing software in blue. These images were used as ground truths to measure the effectiveness of the software. The software was used to output the original image indicating the areas of detected break in red. This allowed for a simple visual comparison between red (software detected) and blue (technician graded). The images were also compared with regard to pixel count.

Clinical Validation

A single-center, one visit study enrolling 29 dry eye and 16 normal subjects was conducted. All subjects were enrolled based on qualifying eyes, meaning a subject could contribute 1 or 2 eyes. Qualifying eyes included 49 eyes

from the 29 dry eye subjects and 29 eyes from the 16 normal subjects, for a total of 78 qualifying eyes. Qualifying eyes for the dry eye subjects were based on three inclusion criteria: a forced-stare TFBUT of ≤ 5 seconds in at least one eye; a corneal fluorescein staining score ≥2 (0-4 point Ora scale) in at least one region of the eye; and a reported history of dry eye disease or ocular symptomatology with the desire to use artificial tears. Normal subjects were excluded if they had a history of dry eye, irritation, or any other ocular problems, wore contact lenses or had LASIK eye surgery, or habitually used artificial tears or tear substitutes. To ensure that normal subjects were largely free of keratitis, qualifying eyes for the normal subjects must also have had a ≤ 1.5 staining score in each region of both eyes (0-4 point Ora scale). A staining score of ≥ 2 in any region of any eye was exclusionary. In addition to forced-stare tear film breakup time, fluorescein staining evaluations, all enrolled subjects were measured by the OPI 2.0 System. Additionally, all dry eye subjects and a random sampling of four normal subjects underwent Schirmer's test evaluations.

Three additional analyses were performed looking at worst eye only, meaning that each subject only contributed a single eye to each analysis. The eye was defined as "worst" using three separate criteria in three independent analyses; first by looking at total staining score, second by looking at forced-stare TFBUT, and finally by looking at MBA. Finally, dry eye and normal groups were compared with respect to variability. Ratios of standard deviation were used for

Schirmer's and staining, while ratios of coefficients of variation were used for forced-stare TFBUT, IBI, MBA, and OPI 2.0.

Forced-stare tear film breakup time was evaluated by instilling sodium fluorescein solution (5µl, 2% preservative-free) into the inferior conjunctival cul-de-sac of each eye, and the subject was asked to blink several times to mix the fluorescein with their tear film. The subject was then asked to blink twice and then stare without blinking for as long as possible. The examiner monitored the integrity of the tear film through a slit-lamp biomicroscope with an 8 mm scanning beam (using an excitation blue filter and a barrier Wratten #12 yellow filter), and measured the time from eye opening to the first appearance of micelles with a stopwatch. The eyes were evaluated sequentially (OD, OS). Two measurements were taken and averaged unless the two measurements were both less than 10 seconds and differed by more than 2 seconds, in which case a third measurement was taken and the two closest of the three were averaged.

Following the traditional clinical assessments, OPI 2.0 System measurements were taken. The examiner instilled sodium fluorescein solution (5µl, 2% preservative-free) into the inferior conjunctival cul-de-sac of each eye and the subject was asked to blink several times to mix the fluorescein with the tear film.   While the subject performed a standard visual task (watching a documentary on television from a five foot viewing distance), the eye was recorded using a digital video camera (EYECAP IM 900 camera System) at 8x magnification at a rate of 15 frames per second (FPS) through a slit-lamp

biomicroscope using an excitation blue filter and a barrier Wratten #12 yellow filter. A minimum of one minute of continuous data was recorded for each eye with approximately thirty seconds between recordings of the two eyes.   The eyes were recorded from right (OD) to left (OS). Subsequently, a computer program analyzed the cornea on a frame-by-frame basis and provided BA for each IBI during the one minute video. From this analysis, MBA and OPI 2.0 were calculated and analyzed. The software also provides a measurement of TFBUT; however for the purposes of this paper, this data was not analyzed.

Statistical Analysis

The comparison between independent dry eye and normal qualifying eyes was based on 78 eyes (dry eye = 49, normals = 29). Normal linear models estimated by generalized estimating equation (GEE) methods were used for staining scores, and Schirmer's scores. Gamma multiplicative models, also estimated by GEE methods, were used for MBA, IBI, OPI 2.0 and forced-stare TFBUT.

These models provided estimates for group means, differences of means for linear models, and ratios of means for multiplicative linear models. Corresponding 95% confidence intervals, and P-values for tests of equality, were calculated. All models were fit using the genmod procedure of SAS version 9.2.("SAS Institute Inc. 2009. SAS OnlineDoc® 9.2. Cary, NC: SAS Institute Inc,")

Results

Verification

The software analysis was able to correctly identify the area of exposure in a set of artificially constructed images created to mimic the visual properties of actual clinical images captured using fluorescein staining videography. The OPI 2.0 System false positive and false negative errors were dependent on the given parameters (density, p=0.004; dispersion, p=0.038; brightness, p<0.001) of the real images (**Figure 3.2**).

Figure 3.2. *The OPI 2.0 System false positive and false negative errors and verification of the software analysis* (images of LLL (2a), HLH (2b), HHH (2c) shown) with designated artificial eye on the left and OPI 2.0 System output with the areas of detected simulated tear film breakup in red on the right.

Density (p=0.004)[1]	Dispersion (p=0.038)[1]	Brightness (p<0.001)[1]	False Negative	False Positive	Total False	Total Pixels	% Error Rate
L	L	L	0	18	18	404811	0.0044
L	L	H	0	4	4	404670	0.0010
L	H	L	0	10	10	404670	0.0025
L	H	H	1	6	7	404670	0.0017
M	M	M	0	6	6	404817	0.0015
H	L	L	5	9	14	404806	0.0035
H	L	H	0	0	0	404806	0.0000
H	H	L	1	2	3	404670	0.0007
H	H	H	0	0	0	404670	0.000

[1]The OPI 2.0 System false positive and false negative errors were dependent on the given parameters.

A.
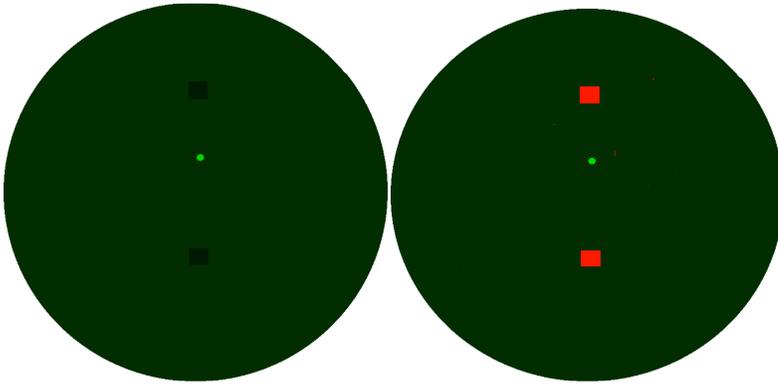
B.
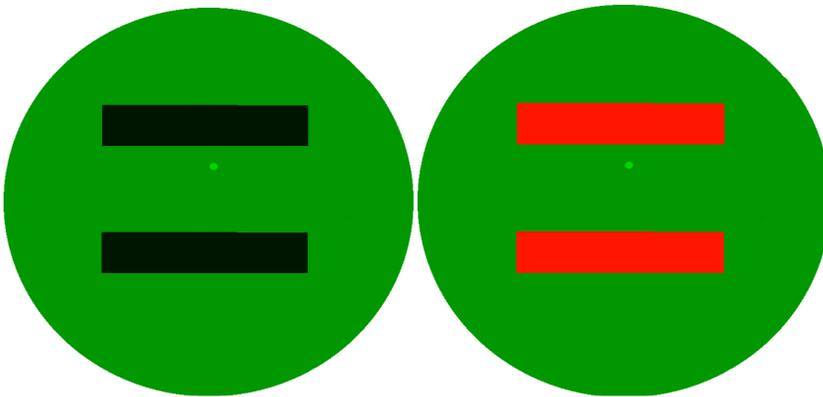
C.
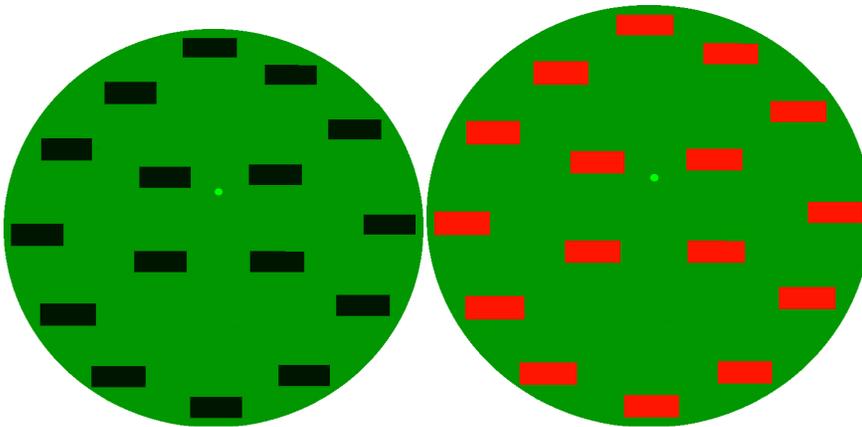
For all nine images, out of 3,642,590 pixels, there were a total of 62 false errors,

yielding a 99.9983% accuracy rate. Seven of the errors were false negatives

while 55 were false positives. In the artificial eye designated LLL (low density,

low dispersion, low brightness, **Figure 3.2a**), the OPI 2.0 System detected the

greatest number of false positive and false negative pixels with a total of 18, zero

of which were false negative and all 18 of which were false positive. In the

artificial eyes designated HLH (high density, low dispersion, high brightness,

Figure 3.2b) and HHH (high density, high dispersion, high brightness, **Figure**

3.2c), the OPI 2.0 System detected the least number of false positive and false

negative pixels, both with a total of zero.

Validation

The software analysis was able to correctly identify the area of exposure in a set

of video images collected (**Figure 3.3**).

Figure 3.3. *The OPI 2.0 System false positive and false negative errors and verification of the software analysis using actual videos collected* (image of HHL (3a) shown) with the technician graded image in blue on the left and OPI 2.0 System output with the areas of detected simulated tear film breakup in red on the right.

Density	Dispersion	Brightness	False Negative	False Positive	Total False	Total Pixels	% Error Rate
L	L	L	8	0	8	325620	0.0025
L	L	H	0	0	0	396360	0.0000
L	H	L	1852	983	2835	364704	0.7773
L	H	H	1169	769	1938	256432	0.7558
M	M	M	1654	1021	2675	444730	0.6015
H	L	L	740	6168	6908	313040	2.2067
H	L	H	1561	4044	5605	282400	1.9848
H	H	L	5550	7307	12857	444136	2.8948
H	H	H	1516	4386	5902	337640	1.7480

A.
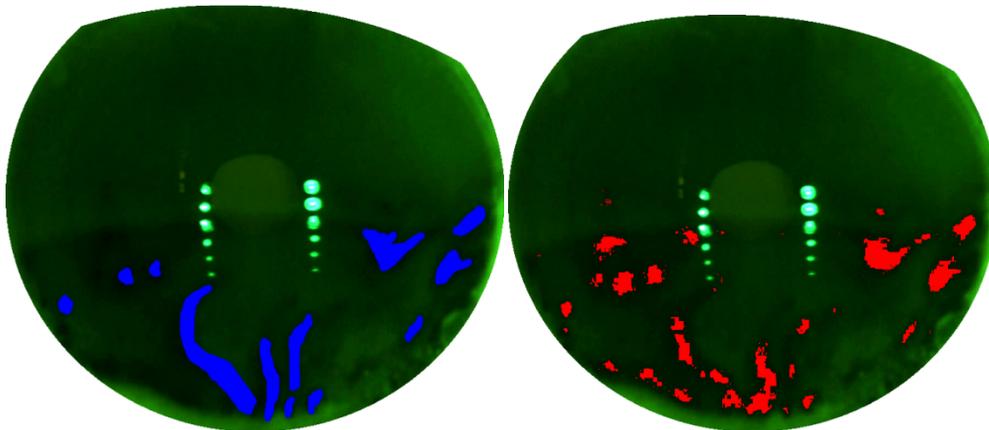
For all nine images, out of 3,165,062 pixels, there were a total of 38,728 false

errors, yielding a 98.7764 % accuracy rate. Fourteen thousand and fifty (14, 050)

of the errors were false negatives while 24,678 were false positives. In the

technician graded eye designated HHL (high density, high dispersion, low

brightness, **Figure 3.3a**), the OPI 2.0 System detected the greatest number of false

positive and false negative pixels with a total of 12,857, of these, 5,550 were false

negatives and 7,307 were false positives. While this error rate was the highest at

2.8948%, it can likely be attributed to a discrepancy in the inaccuracy of the

technician graded image. In the technician graded eye designated LLH (low

density, low dispersion, high brightness) the OPI 2.0 System detected the least

number of false positive and false negative pixels with a total of zero.

Clinical Validation

The mean ages for the dry eye (n=29) and normal (n=16) subjects with qualifying

eyes were 59.08 and 34.03 years, respectively. A total of 49 and 29 eyes qualified

for the dry eye and normal subjects, respectively. Three additional analyses were

performed looking at the worst eye of both dry eye and normal subjects using

three separate criteria: worst eye based on staining, worst eye based on forced-

stare TFBUT, and worst eye based on MBA. Each subject only contributed a

single eye to each analysis, for a total of 45 eyes per analysis (dry eye = 29,

normal = 16). For the variability analysis, ratios of standard deviation were used

for staining, while ratios of coefficients of variation were used for forced-stare

TFBUT, IBI, MBA, and OPI 2.0.

All Qualifying Eyes

The Schirmer's score means for the dry eye qualifying eyes and for the four

qualifying eyes of the randomly selected normal subjects were 11.938 and 21.000

mm, respectively, for a ratio of 0.568 (p=0.330). The forced-stare TFBUT means

for the dry eye and normal qualifying eyes were 2.599 and 10.908 seconds,

respectively, for a ratio of 0.238 (p<0.001). **Figure 3.4** shows histograms for both

dry eye and normal qualifying eyes.

Figure 3.4. *Average forced-stare TFBUT for dry eye and normal qualifying eyes.*
Observed (yellow) and modeled (green, lognormal) histogram.
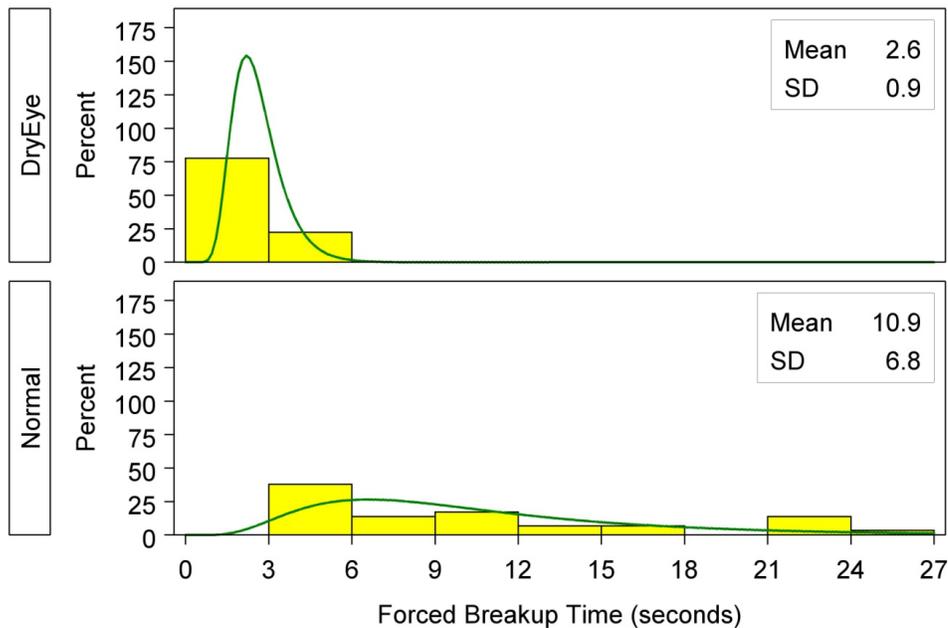
The staining score means of the entire cornea for the dry eye and normal

qualifying eyes were 1.983 and 0.241, respectively, for a ratio of 8.215 (p<0.001).

The staining score means of the superior region of the cornea for the dry eye and

normal qualifying eyes were 1.878 and 0.207, respectively, for a ratio of 9.075

(p<0.001). The staining score means of the central region of the cornea for the

dry eye and normal qualifying eyes were 1.765 and 0.103, respectively, for a ratio of 17.065 (p<0.001). The staining score means of the inferior region of the cornea for the dry eye and normal qualifying eyes were 2.306 and 0.414 respectively, for a ratio of 5.573 (p<0.001).

The IBI means for the dry eye and normal qualifying eyes were 10.710 and 7.114 seconds, respectively, for a ratio of 1.506 (p=0.098). The MBA (mean percent of the cornea exposed) of the entire cornea for the dry eye and normal qualifying eyes was 0.232 and 0.040, respectively, for a ratio of 5.882 (p<0.001). The MBA of the central region of the cornea for the dry eye and normal qualifying eyes was 0.052 and 0.014, respectively, for a ratio of 3.877 (p=0.029). The MBA of the inferior region of the cornea for the dry eye and normal qualifying eyes was 0.137 and 0.013, respectively, for a ratio of 10.730 (p<0.001). The MBA of the superior region of the cornea for the dry eye and normal qualifying eyes was 0.043 and 0.013, respectively, for a ratio of 3.256 (p=0.023). **Figure 3.5** shows histograms for both dry eye and normal qualifying eyes. The OPI 2.0 (in units of mean % cornea exposed/second) represents the fraction of corneal surface at risk or exposed. The OPI 2.0 of the entire cornea for the dry eye and normal qualifying eyes was 0.039 and 0.006, respectively, for a ratio of 6.111 (p<0.001). The OPI 2.0 of the central cornea for the dry eye and normal qualifying eyes was 0.009 and 0.002, respectively, for a ratio of 3.947 (p=0.061). The OPI 2.0 of the inferior cornea for the dry eye and normal qualifying eyes was 0.025 and 0.002, respectively, for a ratio of 15.537 (p<0.001). The OPI 2.0 of the

superior cornea for the dry eye and normal qualifying eyes was 0.005 and 0.002, respectively, for a ratio of 1.946 (p=0.120). **Figure 3.6** shows histograms for both dry eye and normal qualifying eyes. **Figure 3.7** shows mean MBA versus IBI for both dry eye and normal qualifying eyes.

Figure 3.5. *MBA for dry eye and normal qualifying eyes.* Observed (yellow) and modeled (brown, lognormal) histogram.
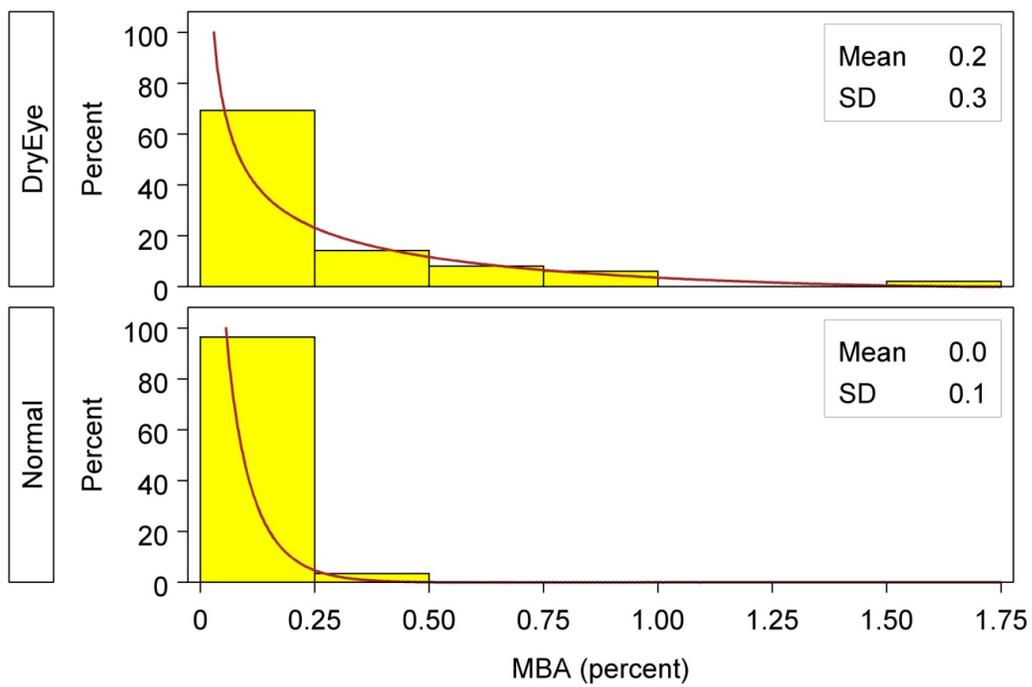
Figure 3.6. *OPI 2.0 for dry eye and normal qualifying eyes.* Observed (yellow) and modeled (black, lognormal) histogram.
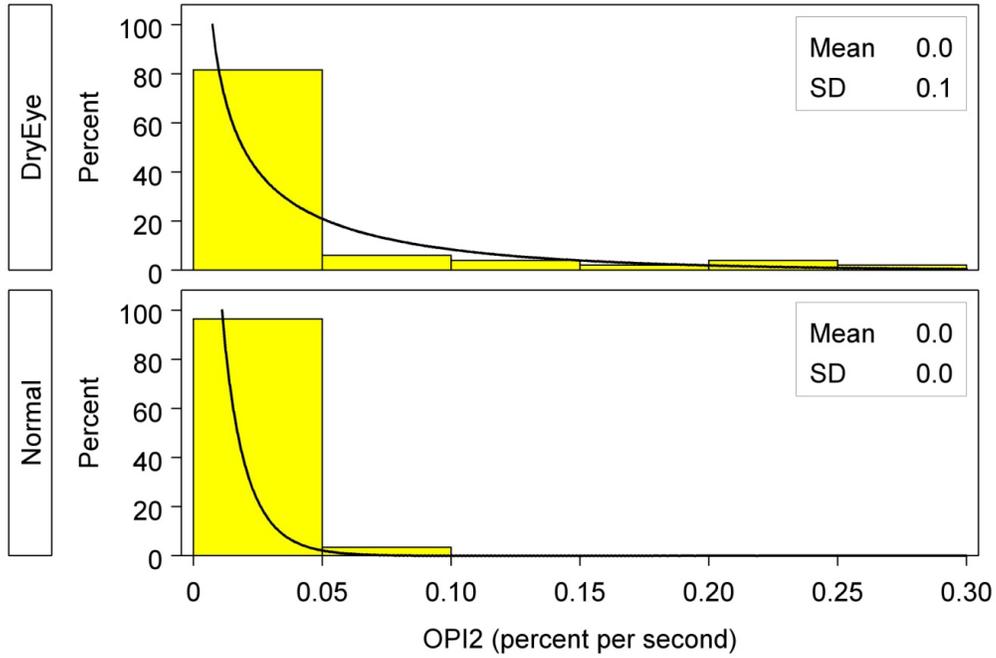
Figure 3.7. *MBA (mean % of the cornea exposed) versus IBI for dry eye (blue) and normal (red) qualifying eyes.* Figure 7A is shown on a linear scale, while figure 7B is shown on a logarithmic scale.

A. B.

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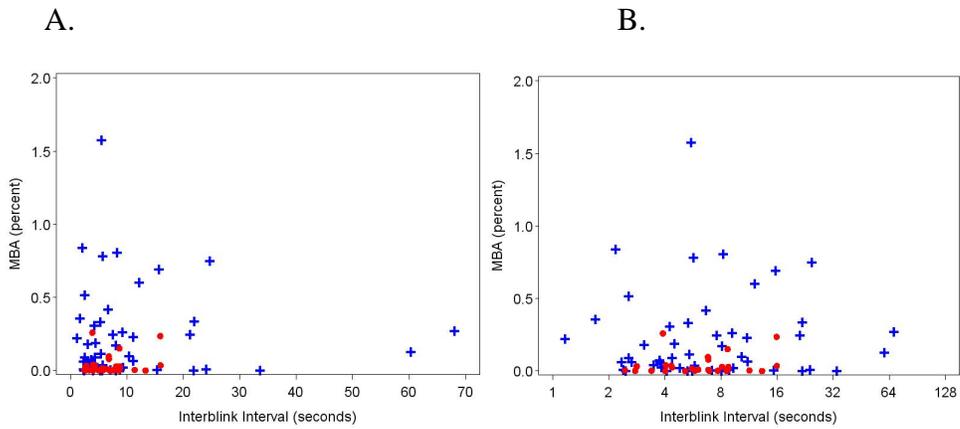
Worst Qualifying Eye

Results for worst qualifying eye based on staining, forced-stare TFBUT, and

MBA are numerically similar to the analysis for all qualifying eyes. The staining

scores means for the entire, central, inferior, and superior cornea were statistically

significant for dry eye and normal worst eyes based on staining, forced-stare

TFBUT, and MBA (**Table 3.1**). The forced-stare TFBUT means were

statistically significant for dry eye and normal worst eyes based on staining,

forced-stare TFBUT, and MBA (**Table 3.1**).

Table 3.1. *Mean staining scores for the entire, central, inferior, and superior cornea for dry eye and normal worst eyes based on staining, forced-stare TFBUT, and MBA*

	Staining			Forced-Stare TFBUT			MBA		
	Dry Eye	Normal	p-value	Dry Eye	Normal	p-value	Dry Eye	Normal	p-value
Staining (all)	2.006	0.312	<0.001	1.902	0.240	<0.001	1.937	0.250	<0.001
Staining (superior)	1.914	0.313	<0.001	1.810	0.250	<0.001	1.828	0.219	<0.001
Staning (central)	1.759	0.125	<0.001	1.672	0.125	<0.001	1.741	0.062	<0.001
Staining (inferior)	2.345	0.500	<0.001	2.224	0.344	<0.001	2.241	0.469	<0.001
Forced-stare TFBUT	2.748	9.844	<0.001	2.393	9.450	<0.001	2.700	11.312	<0.001

The MBA of the entire and inferior cornea was statistically significant for dry eye

and normal worst eyes based on staining, forced-stare TFBUT, and MBA (**Table**

3.2). The OPI 2.0 of the entire and inferior cornea was also statistically

significant for dry eye and normal worst eyes based on staining, forced-stare

TFBUT, and MBA (**Table 3.2**).

Table 3.2. *MBA and OPI 2.0 calculations for the entire, central, inferior, and superior cornea for dry eye and normal worst eyes based on staining, forced-stare TFBUT, and MBA*

	Staining			Forced-Stare TFBUT			MBA		
	Dry Eye	Normal	p-value	Dry Eye	Normal	p-value	Dry Eye	Normal	p-value
MBA (all)	0.227	0.055	0.003	0.210	0.042	0.001	0.300	0.065	<0.001
MBA (central)	0.035	0.021	0.425	0.034	0.022	0.471	0.053	0.023	0.206
MBA (inferior)	0.152	0.019	0.005	0.155	0.005	<0.001	0.190	0.022	<0.001
MBA (superior)	0.040	0.014	0.217	0.021	0.016	0.691	0.057	0.021	0.104
OPI 2.0 (all)	0.039	0.008	0.003	0.040	0.007	0.002	0.049	0.010	<0.001
OPI 2.0(central)	0.006	0.004	0.534	0.006	0.004	0.590	0.009	0.004	0.307
OPI 2.0 (inferior)	0.029	0.002	<0.001	0.031	0.001	<0.001	0.034	0.003	<0.001
OPI 2.0 (superior)	0.004	0.002	0.399	0.002	0.003	0.947	0.006	0.003	0.245

Variability Analysis

Generally, dry eye qualifying eyes showed greater variability than normal

qualifying eyes; typically dry eyes were twice as variable (**Table 3.3**).

Table 3.3. *Dry eye and normal groups compared with respect to variability.* Ratios of standard deviation were used for Shirmer's and staining, while ratios of coefficients of variation were used for forced-stare TFBUT, IBI, MBA, and OPI 2.0.

	Dry Eye	Normal	Ratio	p-value
Schirmer's	8.67	15.06	0.58	0.078
Staining (all)	0.56	0.33	1.67	0.005
Staining (superior)	0.58	0.37	1.59	0.010
Staining (central)	1.00	0.31	3.23	<0.001
Staining (inferior)	0.65	0.48	1.35	0.092
Forced-Stare TFBUT	0.34	0.63	0.54	<0.001
IBI	1.11	0.53	2.09	0.001
MBA (all)	8.72	4.29	2.03	0.282
MBA (central)	8.45	3.10	2.73	0.094
MBA (inferior)	10.99	2.36	4.66	0.010
MBA (superior)	7.00	3.43	2.04	0.226
OPI 2.0 (all)	4.08	1.59	2.57	0.022
OPI 2.0 (central)	2.05	0.93	2.19	0.008
OPI 2.0 (inferior)	4.26	0.78	5.45	<0.001
OPI 2.0 (superior)	1.76	1.18	1.50	0.172

This is also demonstrated graphically in **Figure 3.7**. The dry eye qualifying eyes

had higher variability for MBA and IBI while the normal qualifying eyes were

clustered along the x-axis. The coefficients of variations for MBA for the entire

cornea of dry eye and normal qualifying eyes were 8.72 and 4.29, respectively,

for a ratio of 2.03 (p=0.282). The coefficients of variations for the OPI 2.0 of the

entire cornea for dry eye and normal qualifying eyes were 4.08 and 1.59,

respectively, for a ratio of 2.57 (p=0.022).

Discussion

Our enhanced understanding of the complexities involved with tear film breakup

and blink physiology led to an alternative method for the evaluation of ocular

surface protection under normal visual conditions. Although forced-stare TFBUT has been a standard diagnostic tool for over 40 years, it does not provide sensitive information about the overall health of the tear film, namely what occurs after the break in the tear film. As such, the OPI 2.0 System implements fully automated software algorithms which provide a real-time measurement of corneal exposure (breakup area, BA) for each interblink interval (IBI) during a one minute video. From this system, MBA and OPI 2.0 are calculated and analyzed to garner a more complete picture of ocular surface health. The retrospective manual analysis originally used, however, required numerous technician hours to manually grade the area of corneal coverage. The development of the software analysis allows for a frame-by-frame analysis of percent of corneal area exposed and utilizes computer programs to increase the speed of analysis. The computer program minimizes human error or bias and achieves the outcomes in a more precise manner.

The OPI 2.0 System was able to distinguish between a group of pre-defined dry eye and normal subjects by way of both MBA and OPI 2.0 in statistically significant fashions. Utilizing the software analysis allows for much more precise calculations of MBA and OPI 2.0 than the manual analysis. This can be attributed to the fact that grading for the manual analysis was made based on a binary evaluation of breakup within each region, where a given region was considered to have breakup in that area regardless of the actual extent of exposure. In contrast, the software analysis provides an actual pixel count of BA,

which in turn affords a more precise assessment. Additionally, the manual analysis provides average measurements of the percentage of cornea exposed for the 1-minute observation period at only time 0 (immediately following the blink), at the time of tear film breakup, and at the maximum level of tear film breakup at the end of the IBI. The software analysis, however, analyzes the cornea on a frame-by-frame basis, accounting for individualized points of breakup area.

The OPI 2.0 System also allows for a transect analysis to calculate regional variation over the cornea by analyzing corneal exposure on the basis of inferior, central and superior regions as well as for the entire cornea. One advantage for the use of a transect is the measurement of tear film dispersion (as defined by the verification section). While the use of a transect is not required because the OPI 2.0 System detects breakup area points individually, the use of a transect allows for the assessment of tear film breakup patterns by region. The evaluation on a region-by-region basis parallels other clinical assessments such as staining grading. On an aggregate basis, the results of this study suggest that there may be a relationship between MBA and staining, as an increase in MBA of the dry eye population was consistent with higher staining scores. The results of this study also indicate that certain regions of breakup, in particular the inferior region, may be important indicators of dry eye. The worst eye analysis confirmed our interest in the inferior region of the cornea as a key indicator of dry eye, although further research is warranted.

While the goals of this study were to verify and validate the software analysis, the OPI 2.0 System may also be used to classify dry eye patients into subgroups. Dry eye patients are largely variable, due in part to varying disease states, diurnal variations, extensive visual tasks or environmental stressors that may exacerbate or influence dry eye signs and symptoms.(Davis, et al., 2006; Karson, et al., 1981; Miljanovic, Dana, Sullivan, & Schaumberg, 2007; Patel, Henderson, Bradley, Galloway, & Hunter, 1991; Walker, Lane, Ousler, & Abelson, 2010)   In this study, dry eye patients were typically twice as variable as normal patients, which may be indicative of various subgroups of dry eye patients based on minimal or significant ocular surface exposure and IBI. These various subgroups may represent underlying variations in disease pathophysiology in addition to a distinct opportunity for advances in potential therapies.

It is evident that forced-stare TFBUT alone does not provide enough information to adequately categorize and assess dry eye patients. The OPI 2.0 System allows us to calculate and analyze MBA and OPI 2.0.   MBA is a global way of assessing the percent of cornea exposed, while OPI 2.0 provides information on tear film stability by factoring the IBI to garner a more complete understanding of overall ocular surface health.   Possible limitations of this study include the small normal population analyzed, the measurement of Schirmer's on only four randomly selected normal subjects, and the conduct of the study without a therapeutic agent. Studies are underway employing the OPI 2.0 System to assess the therapeutic

value of a study drug in a clinical trial setting. Further research to understand the relationship between MBA, OPI 2.0 and potential dry eye subgroups is necessary.

CHAPTER 4

A SINGLE-CENTER STUDY EVALUATING THE EFFECT OF THE CONTROLLED ADVERSE ENVIORNMENT (CAE) ON TEAR FILM STABILITY

Background

Dry eye disease is a term used to describe a collection of disorders with a shared diagnosis of *tear film dysfunction,* leading to decreased visual acuity, ocular pain, burning, and the potential for corneal scarring.("The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007)," 2007) Prevalence is highest in older individuals and in women, as well those who have previously undergone laser vision correction. The most recent reports suggest that moderate to severe dry eye currently affects

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between five and ten million Americans, with estimates of ten times that number world-wide.(Pflugfelder, 2008) Current treatments include artificial tears, tear plugs, or immune-suppressant drugs such as cyclosporine, but these treatments are often ineffective for many dry eye sufferers, and so the unmet need for new therapeutics is significant.

Knowledge of the pathophysiology of dry eye has made considerable progress in recent years, and what was once thought to be a condition due simply to insufficient tear production is now recognized as a multi-factorial collection of diseases. This is a result of significant strides in basic research in ocular surface biology, in combination with improvements in clinical assessment techniques such as corneal staining, blink pattern analysis, and various measures of tear film stability. In two recent papers we described the stepwise development of our improved method of tear film analysis which we have designated as OPI 2.0 System.(Kellerman, et al., 2004; Ousler GW, et al., 1999) This method combines a number of optimized parameters with automated data capture and analysis to generate a more objective, quantitative measure of tear film dynamics. These features have the potential to substantially enhance tear film metrics, and represent a key advance over previous methods of tear film analysis.

Tear film stability and blink behavior are inexorably linked; the tear film is established by the sweeping, squeegee-like action of the lids, and rate and pattern of blinks is, in turn, modulated by feedback input from corneal sensory nerves.("The epidemiology of dry eye disease: report of the Epidemiology

Subcommittee of the International Dry Eye WorkShop (2007)," 2007) Efforts to measure properties of the tear film led to the development of tear film break-up time (TFBUT), a methodology in which subjects are asked to refrain from blinking while an observer monitors the integrity of the tear film.("The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007)," 2007) This "forced stare" approach allowed the first estimates of inherent tear film stability and provided the means to address how different disorders, drug treatments, or environmental conditions might impact the physiochemical properties (and therefore, functional attributes) of the tear film.("The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007)," 2007; G. W. Ousler, et al., 2005) Standards developed with this method were > 10 seconds for normals, and < 10 seconds for subjects with dry eye.("The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007)," 2007; G. W. Ousler, et al., 2005) Subsequent studies have indentified limitations to both the methodologies used to measure TFBUT, as well as the metric itself. For example, reducing and standardizing the quantity of fluorescein used led to a modification of reference values of TFBUT to a mean value of 7 seconds for normal subjects, and 2.5 seconds for those with dry eye.(M. B. Abelson, et al., 2002) Most recently we have developed a measure of tear film stability under conditions of natural, rather than forced stare blinking.(Ousler GW, et al., 1999) By capturing the natural

dynamics of the tear film with automated methods it was possible to extend our studies of the interaction between blinking and TFBUT.(Crampton, et al., 2007b; D'Arienzo, et al., 2007; Kellerman, et al., 2004; G. W. Ousler, 3rd, Hagberg, et al., 2008; Ousler GW, Emory TB, Welch D, & MB., 2002; G. W. Ousler, et al., 2007; Simmons & Vehige, 2007)

Blinking is a reflex function regulated by a combination of autonomic inputs and sensory feedback due to environmental conditions. Blink rate is known to increase under adverse conditions(Ousler GW, et al., 2002) such as those presented by the Controlled Adverse Environment (CAE), an established clinical model that provides a standardized approach to studying investigational treatments of dry eye.(G. W. Ousler, et al., 2005) This model affords a controlled, reproducible environment that challenges the eyes of all patients equally and for the same amount of time, and exacerbates the signs and symptoms of dry eye by regulating humidity, temperature, airflow, lighting conditions, and visual tasking.("The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007)," 2007; Rolando, et al., 2009) Assessment of both blink behavior and tear film stability led to development of the Ocular Protection Index (OPI), a tool used in observational and clinical studies designed to evaluate the interaction between blinking and TFBUT in studies of dry eye.(Crampton, et al., 2007b; D'Arienzo, et al., 2007; G. W. Ousler, 3rd, Hagberg, et al., 2008; Ousler GW, et al., 2002; G. W. Ousler, et al., 2007; Simmons & Vehige, 2007; Torkildsen, et al., 2008) Originally, OPI was

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a ratio of inter-blink interval (IBI) and TFBUT; lower values, and particularly

values less than one were associated with increased risk of keratitis, since, on

average, break-up of the tear film and subsequent corneal exposure would occur

prior to the next blink.(G. W. Ousler, 3rd, Hagberg, et al., 2008) In a recent study

we described an improvement upon this method that employs automation of both

break-up and blink data capture, and use of a revised estimate of corneal surface

exposure based upon the mean break-up area (MBA) rather than time;(Ousler

GW, et al., 1999) we refer to this new metric as OPI 2.0.

 The OPI 2.0 System is designed to evaluate ocular surface protection

under a normal blink pattern and normal visual conditions, and implements fully

automated software algorithms which provide a real-time measurement of corneal

exposure. The system provides a simultaneous measurement of TFBUT, breakup

area (BUA), and IBI. From this, the MBA and OPI 2.0 (MBA/inter-blink interval)

are calculated and analyzed. In an earlier paper we established that this automated

data analysis method provides values comparable to those obtained by manual

analysis of video-captured data with a significantly higher degree of

precision.(Ousler GW, et al., 1999) The OPI 2.0 System demonstrated a robust

ability to distinguish between dry eye and normal subjects, and the software

analysis allowed for precise calculations of ocular surface exposure and ocular

surface protection metrics.

 In this study, we explore the ability of the OPI 2.0 System to identify the

changes and modifications of the tear film after exposing dry eye subjects to the

Controlled Adverse Environment (CAE). A key aspect of the CAE is its utility to distinguish sub-populations of dry eye patients. Subjects challenged by environmental changes (such as those presented by the CAE) normally respond with some degree of physiological compensation, and previous studies have shown that the ability of these mechanisms to adequately compensate for environmental challenges is reduced in those with dry eye.(G. W. Ousler, 3rd, et al., 2002) The nature and extent of the compensatory response will likely be a reflection of the underlying tear film pathology. These compensatory mechanisms, such as changes in blink rate or reflex tearing, are likely to impact properties such as those measured using the OPI 2.0 System. A primary goal in developing new or refined metrics is their use as tools to identify and characterize patient sub-populations, especially in multi-factorial diseases such as dry eye. As the next step in the validation of OPI 2.0 System-based measures, we examined dry eye subjects before, immediately after, and 30 minutes after a 90-minute CAE challenge.

Methods

Inclusion criteria   Subjects were recruited from an existing database of dry eye patients; a total of 33 subjects were enrolled. Criteria for inclusion in the database included a history of dry eye, use of artificial tears, and a Schirmer's test score of < 5 mm in at least 1 eye. Enrolled subjects were at least 18 years of age, had a history of use or desire to use an eye drop for dry eye symptoms within the past 6 months, and had a best corrected visual acuity (BCVA) of +0.7 or better assessed by Early Treatment of Diabetic Retinopathy Study (ETDRS) scale in both eyes. Subjects also had to satisfy each of the following criteria at baseline: a forced-stare TFBUT of < 5 seconds in at least 1 eye; a deficient OPI (< 1) during at least 30% of inter-blink intervals as determined by a trained technician; and a total corneal fluorescein staining score of ≥ 3, based on the sum of the central, superior, and inferior regions of the cornea as anatomically defined by the Ora scale.

Exclusion criteria   Subjects were excluded from the study if they had clinically significant anterior blepharitis in the opinion of the investigator, were diagnosed with on-going ocular infection (bacterial, viral, or fungal) or active ocular inflammation (e.g., follicular conjunctivitis); wore contact lenses in the previous week; had used any eye drop in the 4 hours prior to the study; had previously had laser in situ keratomileusis (LASIK) surgery or any other ocular surgery in the past year; were currently taking any topical ophthalmic prescription or over-the-

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counter (OTC) solutions, artificial tears, gels or scrubs that could not be discontinued for the duration of the trial; had used Restasis® in the previous 30 days; had a systemic disease, or uncontrolled medical condition that could interfere with study measurements or subject compliance; were currently pregnant or nursing; or had received another experimental drug or device within 30 days of visit.

Study Design This was a single-center study, conducted in one visit that included a ninety-minute session in the CAE. Written informed consent was obtained prior to study procedures. Patient-reported and investigator-observed adverse events were captured and monitored for the duration of the study. Fluorescein staining, TFBUT, conjunctival redness, and OPI 2.0 System measurements were conducted at baseline (prior to CAE exposure). Subjects were then exposed to CAE for 90 minutes. Baseline dry eye assessments and OPI 2.0 System measurements were repeated on all subjects immediately following CAE exposure, and again 30 minutes after exposure.

The primary endpoint for this study was MBA prior to CAE exposure compared to MBA immediately and 30 minutes post-CAE exposure, for subjects with pre-CAE MBA values ≥ 0.2. Secondary endpoints included fluorescein staining, TFBUT, and redness prior to CAE compared to fluorescein staining, TFBUT, and redness immediately and 30 minutes post CAE exposure. Additional

secondary endpoints included video-based measurements collected at all 3 time points, including IBI and TFBUT, and palpebral fissure width.

Statistical Analysis Thirty-three subjects were enrolled in the study. Sixty-five of the 66 eyes provided readable videos, and these 65 eyes comprised the complete analysis sample. All 33 subjects in the intent-to-treat population met the requirements of the per-protocol criteria. We also analyzed two sets of subgroups defined by their baseline MBA measure; these were subjects with MBA ≥ 0.2 (n=30) defined by the primary efficacy endpoint, and a second group with an initial MBA ≥ 0.5 (n=19).

Demographic variables (age, sex, duration of dry eye disease) were summarized by means and standard deviations. Variables derived from the one minute videos were MBA – the primary variable – along with OPI2, BR, IBI, and palpebral fissure. These variables were obtained pre-CAE, post-CAE, and 30 minutes post-CAE. Other secondary variables collected at the same time points were BUT, corneal fluorescein staining, and conjunctival redness. In addition, ocular discomfort was collected every 5 minutes during the 90-minute CAE, and these measurements gave rise to additional variables: average discomfort during the CAE, and tearing. We define tearing as the time at which discomfort either reduced or plateaued at a value less than the maximum, based upon previous studies demonstrating that this plateau is associated with a compensatory tearing response.(G. W. Ousler, 3rd, et al., 2002).

For those variables with long right-tailed distributions (including MBA, OPI2, BR, IBI, palpebral fissure, and BUT) we used a gamma multiplicative model to obtain estimates for pre-CAE and post-CAE means, ratios of means, 95% confidence intervals, and P-values for tests of equality. Corneal fluorescein staining and conjunctival redness were analyzed using normal linear models. We used a generalized estimating equation to accommodate for the within-subject correlation between eyes for both models. For the latter purpose, a sandwich variance was used in conjunction with a working independence correlation structure. All models were fit using the GENMOD procedure of SAS version 9.2.("SAS Institute Inc. 2009. SAS OnlineDoc® 9.2. Cary, NC: SAS Institute Inc,")

For variables measured pre- and post-CAE, bivariate relationships were obtained via correlations between change scores (e.g., post-CAE MBA minus pre-CAE MBA versus pre-CAE BR minus post-CAE BR). These change scores were also correlated with variables collected in the CAE (average discomfort, and tearing [score = 1 if there was tearing within 90 minutes, score = 0 otherwise]), and demographic variables of age, duration of dry eye disease, and gender.

Results

In this single visit study, we examined the effect of the CAE on tear film mean breakup area (MBA) for subjects with confirmed dry eye disease. A total of 33 dry eye subjects completed the study with 65 qualified eyes entered into the

analysis algorithm. One video file was corrupted during the course of collection

and could not be included in the analysis. No treatment was administered in the

course of the study.

The demographics of the study populations were generally representative

of the larger population of all dry eye patients.("The epidemiology of dry eye

disease: report of the Epidemiology Subcommittee of the International Dry Eye

WorkShop (2007)," 2007) The study population was 34% men, had a mean age of

64 years and had experienced dry eye disease for an average of 13.4 years; a

similar demographic profile described all of the sub-populations analyzed in this

study, as summarized in **Table 4.1**.

Table 4.1. *Demographics of study populations.*

Subjects	Age ± SD	% Male	Years with Dry Eye	MBA_I
All eyes (n=65)	64.1 ± 10.8	34	13.4 ± 12.4	0.55 ± 0.85
MBA > 0.5 (n=19)	61.9 ± 14.4	21	14.5 ± 14.8	1.51 ± 1.05
MBA > 0.2 (n=35)	64.5 ± 12.2	40	14.2 ± 14.5	0.96 ± 0.98
MBA < 0.5 (n=46)	64.9 ± 9.0	39	13.0 ± 11.4	0.15 ± 0.14
MBA < 0.2 (n=30)	63.6 ± 9.0	27	12.4 ± 9.5	0.06 ± 0.06

The primary endpoint was the change in MBA between the baseline, pre-CAE

values and MBA values determined immediately and 30 minutes after exposure to

the CAE for subjects with initial MBA measures of 0.2 or greater. The change in

MBA for 3 subject populations, including those with initial MBA scores < 0.2, is shown in **Figure 4.1A**.

Figure 4.1. *A Comparison of endpoints before and after CAE.* Graphs include data for the primary efficacy endpoint, MBA in subjects with $MBA_I > 0.2$.

A. Mean Break-up Area

B. Blink Rate

C. Corneal Staining
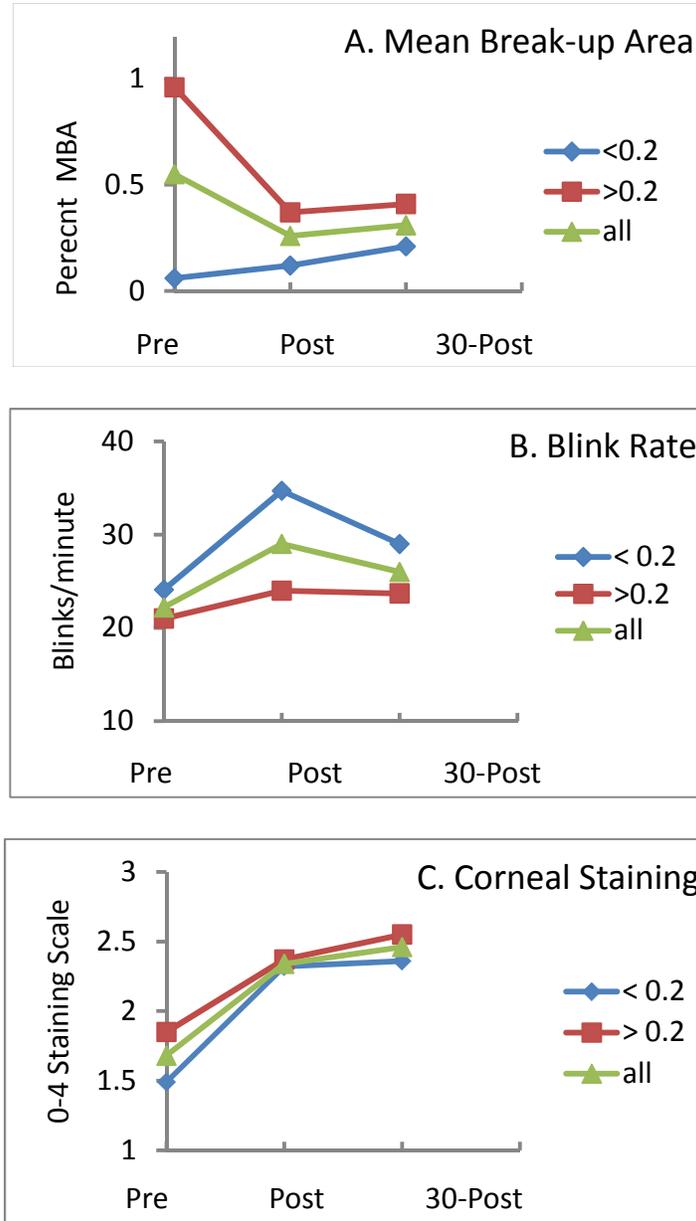
Subject with higher initial MBA are those with more severe dry eye, and we found that in this population, as well as the entire subject population, exposure to the CAE caused a significant decrease in MBA. This figure also shows that for those subjects with less severe dry eye (initial MBA, MBA_I, <0.2), there is a trend toward increased MBA which is not statistically significant. **Figure 4.1B and 4.1C** show comparisons of two additional metrics in these 3 populations; blink rate and corneal staining. The change in blink rate observed over the course of the study is interesting in that while the population as a whole increased significantly, the group with initial MBA > 0.2 did not exhibit this increase (**Figure 4.1B and Table 4.2**). Corneal fluorescein staining is noteworthy for several reasons. First, the difference between those with initial MBA > 0.2 and those < 0.2 is significant ($p < 0.001$), confirming our premise that those with higher MBA scores have more severe dry eye. Second, all subjects show significant increases in staining over the course of the visit, as expected for exposure to the adverse environment of the CAE (**Figure 4.1C, Table 4.2**). Of note, this increase in corneal staining occurs regardless of the change in MBA observed for the various subject populations.

Table 4.2. *CAE effects on measures of dry eye.* Mean values all endpoints, for three measured time-points. Top, all eyes (n=65); bottom, eyes with initial MBA (MBA_I) > 0.2 (n=35). * = p_{val} < 0.001;   † = p_{val} < 0.01 as compared with pre-CAE value.

All eyes (n=65)								
	MBA	Blink rate	Palprebral Fissure	Corneal Staining	Redness	OPI 2.0	TFBUT	IBI
Pre-CAE	0.55	22.21	1.34	1.68	1.53	0.19	3.77	3.6
Post-CAE	0.26*	29.01*	1.24*	2.34*	2.35*	0.08†	3.82	3.29
30 min-Post-CAE	0.31†	26.15†	1.28*	2.46*	2.22*	0.12	4.09	3.35

MBA_I > 0.2 (n=35)								
	MBA	Blink rate	Palprebral Fissure	Corneal Staining	Redness	OPI 2.0	TFBUT	IBI
Pre-CAE	0.96	20.56	1.35	1.85	1.67	0.33	3.89	4.41
Post-CAE	0.37*	24.12	1.25*	2.37*	2.47*	0.11*	4.05	4.74
30 min-Post-CAE	0.41*	23.72	1.28	2.55*	2.41*	0.14*	4.21	4.36

Secondary endpoints included blink rate, palpebral fissure size, corneal staining, conjunctival redness, OPI 2.0, TFBUT, and IBI. The mean values for these parameters are shown in **Table 4.2**; differences and p-values between pre-CAE and post-CAE values are shown in **Table 4.3**.

Table 4.3. *Difference values for primary and secondary endpoints in different subpopulations.* Values represent the change from pre- to post-CAE measures. Subpopulations are based upon the initial mean break-up area (MBA$_I$) measure. Shaded areas highlight statistically significant changes in subpopulations.

	MBA	Blink Rate	Palprebral Fissure	Staining	Redness	OPI 2.0	TFBUT	IBI
ALL N=65	-0.29 (p<0.001)	6.8 (p<0.001)	-0.11 (p<0.001)	0.52 (p<0.001)	0.80 (p<0.001)	-0.11 (p=0.003)	0.17 (p=0.661)	-0.30 (p=0.935)
MBA$_I$ > 0.5 (N=19)	-1.11 (p<0.001)	-1.75 (p=0.488)	-0.08 (p=0.053)	0.65 (p<0.001)	0.71 (p<0.001)	-0.41 (p<0.001)	0.40 (p=0.533)	0.65 (p=0.315)
MBA$_I$ > 0.2 (N=35)	-0.59 (p<0.001)	3.56 (p=0.088)	-0.10 (p<0.001)	0.52 (p<0.001)	0.80 (p<0.001)	-0.21 (p<0.001)	0.17 (p=0.661)	0.03 (p=0.935)
MBA$_I$ < 0.5 (N=46)	0.05 (p=0.216)	10.33 (p<0.001)	-0.12 (p<0.001)	0.67 (p<0.001)	0.87 (p<0.001)	0.02 (p=0.191)	-0. 09 (p=0.666)	-0.70 (p=0.003)
MBA$_I$ < 0.2 (N=30)	0.06 (p=0.026)	10.58 (p<0.001)	-0.11 (p<0.001)	0.83 (p<0.001)	0.85 (p<0.001)	0.02 (p=0.024)	-0. 07 (p=0.778)	-0.70 (p=0.006)

The major finding is the statistically significant decrease in the MBA immediately following CAE exposure. MBA values 30 minutes post-CAE were also significantly lower than baseline, although there was some recovery between the two post-CAE time points. As expected, both fluorescein staining and redness increase with CAE exposure (**Tables 4.2 and 4.3**). Blink rate and palpebral fissure width also decreased significantly across the population, at both post-CAE times. In contrast, mean IBI and TFBUT changed only minimally, and not significantly. **Table 4.3** summarizes the difference scores for all endpoints, and shows the same pattern of significance for primary and secondary endpoints seen in mean value comparisons.

Based upon the pattern of decreased MBA and increased blink rates, we examined a second set of subpopulations that were defined using a higher threshold of initial MBA (> 0.5; n = 19). These subjects had a higher initial

corneal staining score, and exhibited a more pronounced decrease in MBA in response to CAE exposure (**Table 4.3, Figure 4.2A**). Analysis of these sub-populations (initial MBA values greater or less than either 0.2 or 0.5) suggested that the MBA metric may provide the means to distinguish between individuals who respond in the CAE with differing compensatory mechanisms. Comparison of subjects with either high or low MBA shows that there is a clear, statistically significant difference in the way these two groups respond to the CAE. **Figures 4.1A and 4.2A** show that the decrease in MBA observed in full study population is due to the decrease in this more severe subpopulation; note that for the 2 lower MBA_I groups the mean value increases slightly over the course of the CAE exposure (**Table 4.3**). A second distinction between those with low versus high initial MBA values is shown in **Figures 4.1B and 4.2B**; despite a decrease in MBA during the CAE, subjects with high MBA values show no change in blink rate, while those with low initial MBA values increase their blink rate in the CAE by almost 50% (**Table 4.3**). Reduction in palpebral fissure accompanies this increase in blink rate.

Tear film break-up time has been used as a standard metric in dry eye studies for many years, but results from this study suggest that it does not reflect the changes in dry eye signs and symptoms resulting from CAE exposure**. Figure 4.2C and Tables 4.2 and 4.3** show that none of the populations examined in this study show a significant change in TFBUT, despite the fact that all other metrics

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associated with dry eye, including corneal staining, ocular redness, and

discomfort (not shown) increase over the time course of the CAE.

Figure 4.2. *Subpopulation Comparisons.* Mean values pre, post and 30-min post CAE for two alternative sub-populations. $MBA_I > 0.5$ (n=19) and $MBA_I < 0.5$ (n=46).
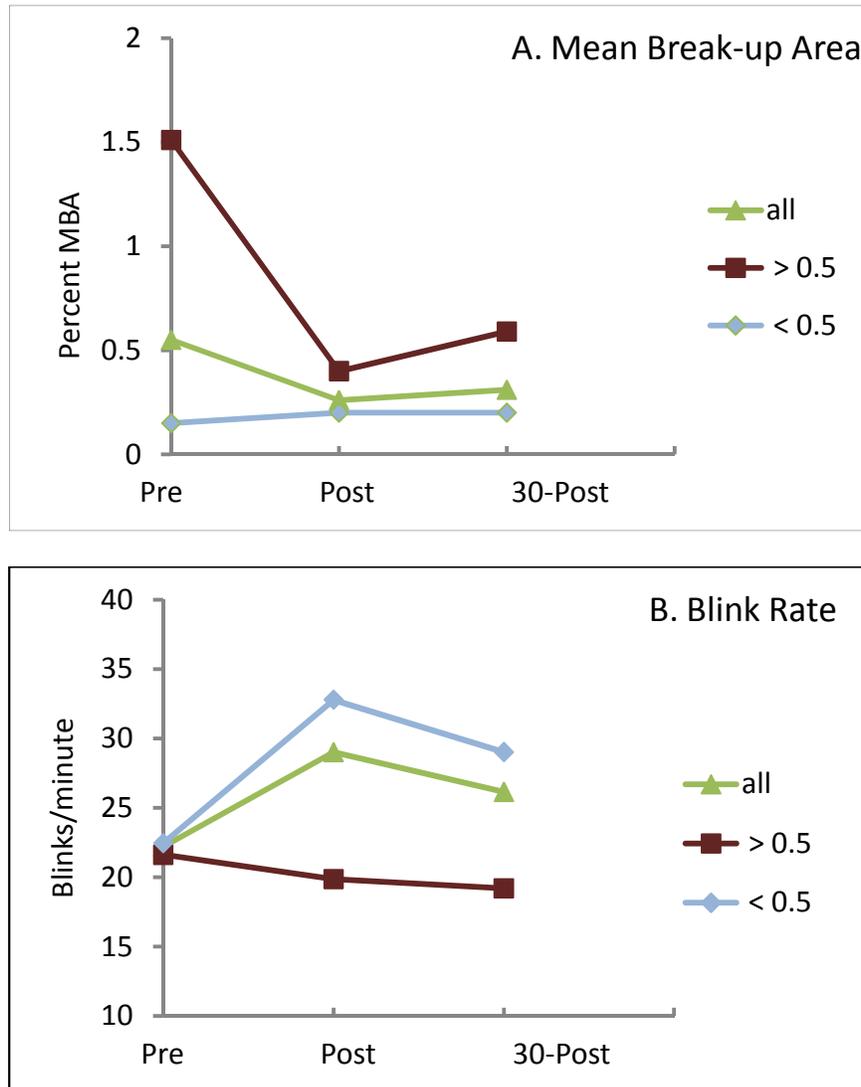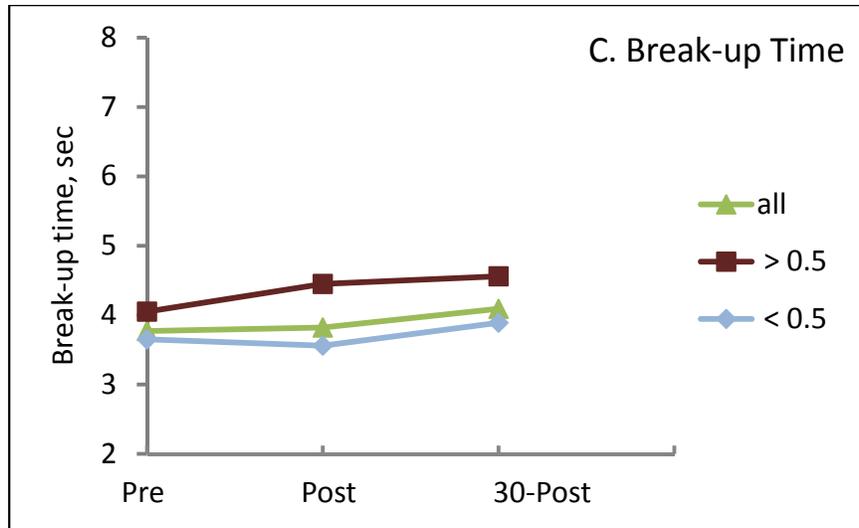
We next did a correlation analysis to test whether specific endpoints might show an association with MBA changes; this data is summarized in **Table 4.4** for the population as a whole, and for the more severe subjects ($MBA_I > 0.2$).

Table 4.4. *Pearson correlation coefficients between MBA and secondary endpoint measures.* Significant values ($p < 0.05$) are shaded.

	Tearing	Blink rate	Fissure	Staining	Redness	TFBUT	IBI
$MBA_I > 0.2$ (n=35)	0.336 (p=0.048)	0.399 (p=0.0174)	0.203 (p=0.242)	- 0.194 (p=0.234)	0.263 (p=0.127)	-0.162 (p=0.353)	- 0.344 (p=0.043)
All subjects (n=65)	0.177 (p=0.159)	0.338 (p=0.0059)	0.141 (p=0.260)	0.0169 (p=0.894)	0.183 (p=0.145)	- 0.151 (P=0.230)	- 0.341 (P=0.005)

Of the endpoints analyzed, only tearing and blink rate (and the related parameter, IBI) were significantly correlated with MBA measures. In addition, all endpoints except IBI showed a positive correlation with MBA in the full study population.

When this same analysis was applied to the high and low MBA subpopulations, the correlation between blink rate and MBA is restricted to the high MBA population, while subjects in the low MBA population exhibit an inverse correlation these two parameters. This distinction indicates that the two subpopulations may be responding to the CAE with different compensatory mechanisms. Taken together, our results suggest that subjects in the low MBA group ameliorate the effects of the CAE, at least in part, by increasing their blink rate, while the high MBA group does not. Despite this lack of change in compensatory metrics, subjects in the high MBA group exhibit a significant decrease in MBA and therefore are clearly responding to the CAE in some way. Throughout the course of CAE exposure, subjects were asked to rate their ocular discomfort (not shown). The values for all groups display a slow, consistent increase in scores over the time course of the CAE, and none of differences between group scores are statistically significant at any time point. This suggests that discomfort alone cannot explain the differences in responses seen in the two groups.

Discussion

Dry eye disease is an exceeding complex disease because of the variety of etiologies and the overlapping, interacting sensory elements and response mechanisms in place designed to maintain an optimally tuned tear film. Identification of endpoints that are both meaningful and measurable has been

problematic. The study presented here represents one step in the process of establishing robust quantitative metrics for clinical studies. In particular, our goal has been to identify measures that are responsive in clinical models designed to replicate the disease process, and are capable of distinguishing between the subpopulations characteristic of this disease. The data presented here establish that the OPI 2.0 system and the measure of MBA can provide the assessment tools necessary to meet this goal.

The key finding of this study is that MBA is significantly decreased in dry eye subjects following CAE exposure, and this decrease identifies a subpopulation of dry eye subjects. In contrast, the traditional metric of tear film stability, TFBUT, is not significantly altered by CAE exposure. Patients also exhibit significant increases in corneal staining, ocular redness, and decreases in palpebral fissure width that are all characteristic of dry eye disease. Thus, in a clinical model which reliably elicits signs and symptoms of dry eye disease, MBA provides a useful new metric that is superior to TFBUT.

Patients completing the CAE exposure fell into two groups. The first group, which comprised about 70% of all subjects, was distinguished by a relatively stable MBA which was maintained in part by an increase in blink rate and a decrease in palpebral fissure width. These changes indicated they were able to respond to the environmental challenge with these (and perhaps other) mechanisms in order to maintain a relatively constant corneal surface exposure, as measured by MBA. The second, smaller group of subjects began the study with

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levels of corneal staining similar to the low MBA group despite a baseline break-up area that was 10 fold higher. Subjects in this group responded to the CAE by lowering their MBA more that 3-fold during the course of the CAE exposure. While we speculate that this response employed some combination of increases in tearing, mucin secretion, or meibum expression, our study did not examine these specific tear film parameters. The net effect of their response however is evidenced by the fact that corneal staining between the two groups was comparable. Future studies will benefit from inclusion of techniques that can monitor changes in these tear film components in the course of CAE exposure.

Our study has direct impact on the design of therapeutic development strategies going forward. First, we have provided direct evidence that break-up area, and not break-up time, is the more valuable parameter in studies of induced dry eye disease. Second, we have established that through the metrics of the OPI 2.0 system we can distinguish subpopulations of subjects who are likely to require different therapeutic strategies for successful amelioration of their dry eye signs and symptoms.

CHAPTER 5

SUMMARY, RECOMMENDATIONS FOR FUTURE
RESEARCH AND CONCLUSION

Summary

In this research two new methods of evaluating Tear Film Stability

simultaneously with IBI were were developed. The first, a manual method, of

measuring ocular exposure in the context of a natural blink pattern through

analysis of the variables tear film break-up time (TFBUT), inter-blink

interval (IBI) and tear film break-up area (BUA). The second an automated

method called The OPI 2.0 System implements fully automated software

algorithms which provide a real-time measurement of corneal exposure

(breakup area, BA) for each interblink interval (IBI) during a one minute

video. Finally the validated OPI 2.0 System is used to evaluate subject Tear

Film Stability when exposed to the Controlled Adverse Environment (CAE)

for inducing the signs and symptoms of Dry Eye disease.

In the second chapter the manual methodology is tested: The new

methodology (video capture manual analysis, VCMA) which involves

retrospective analysis of video data of fluorescein-stained eyes, taken through

a slit lamp while the subject watches television, provides TFBUT, and BUA

for each IBI during the 1 minute video. Traditional methodology measures

TFBUT and IBI separately; TFBUT under forced-stare conditions, as

measured by an examiner using a stopwatch, and IBI while the subject

watches television. The Forced-Stare and VCMA methods were directly

compared in the same set of dry eye subjects. The VCMA method was

evaluated for the ability to discriminate between dry eye subjects and normal

subjects. The VCMA method was further evaluated in the dry eye subjects

for the ability to detect a treatment effect before, and 10 minutes after,

bilateral instillation of an artificial tear solution

Results: Ten normal subjects and 17 dry eye subjects were studied. In the dry

eye subjects, the two methods differed with respect to mean TFBUTs (5.82 sec,

FS, and 3.98 sec, VCMA, $P = 0.002$). The FS variables alone (TFBUT, IBI) were

not able to successfully distinguish between the dry eye and normal subjects,

whereas the additional VCMA variables, both derived and observed (BUA,

BUA/IBI, breakup rate), were able to successfully distinguish between the dry eye

and normal subjects in a statistically significant fashion. TFBUT ($P = 0.034$) and

BUA/IBI ($P = 0.001$) were able to distinguish the treatment effect of artificial

tears in dry eye subjects.

Conclusion: The VCMA methodology provides a clinically relevant analysis of

tear film stability measured in the context of a natural blink pattern.

In the third chapter the OPI 2.0 System is evaluated for its ability to distinguish

between dry eye and normal subjects, and more accurately identify breakup area.

The OPI 2.0 System is utilized to calculate and analyze, the mean breakup area

(MBA) and the OPI 2.0, mean breakup area/interblink interval (MBA/IBI). In

order to verify and validate the OPI 2.0 System, a series of artificial images and still image frames captured during an actual clinical session using fluorescein staining videography were analyzed. Finally, a clinical validation process was completed to determine the effectiveness and clinical relevance of the OPI 2.0 System to differentiate between dry eye and normal subjects.

Results: Software analysis verification conducted in a set of artificially constructed images and in actual videos both saw minimal error rates. MBA and OPI 2.0 calculations were able to distinguish between the qualifying eyes of the dry eye and normal subjects in a statistically significant fashion (p<0.001 and p<0.001, respectively). As expected, the dry eye subjects had a higher MBA and OPI 2.0 than the normal subjects (0.232, dry eye; 0.040, normal and 0.039, dry eye; 0.006, normal, respectively). Results for the worst eyes and all qualifying analyses based on staining, forced-stare TFBUT, and MBA were numerically similar.

Conclusion: The OPI 2.0 System accurately identifies the amount of MBA on the cornea and represents an efficient, clinically relevant measurement of the pathophysiology of the ocular surface.

In the fourth chapter we use the OPI 2. 0 System to evaluate changes in tear film stability with respect to other Dry Eye in the Controlled Adverse Environment (CAE) model of dry eye disease. Thirty-three dry eye subjects completed a single-center, one visit, pilot, CAE study. The primary endpoint was mean breakup area

(MBA) as assessed by the OPI 2.0 system. Secondary endpoints included corneal fluorescein staining, tear film break-up time, and OPI 2.0 System measurements. Subjects were also asked to rate their ocular discomfort throughout the CAE. Baseline dry eye endpoints and OPI 2.0 System metrics were measured at baseline, immediately following a 90 minute CAE exposure, and again 30 minutes after exposure.

Results: MBA showed a statistically significant decrease between the post-CAE measurements and the baseline measure. The decrease is relatively specific to those patients with moderate to severe dry eye, as measured by baseline MBA. Secondary endpoints including palpebral fissure size, corneal staining, redness, and OPI 2.0 also show significant changes in comparisons of pre- to post-CAE measurements. There were also significant correlations observed between MBA, blink rate, and palpebral fissure size. Comparison of MBA responses allowed us to identify sub-populations of subjects that exhibited different compensatory mechanisms in response to CAE challenge. Of note, none of the measures of tear film break-up time showed statistically significant changes or correlations in pre- versus post-CAE measures.

Conclusion: This pilot study confirms that the tear film metric MBA can detect changes in the ocular surface induced by a controlled adverse environment, and that these changes are correlated with other, established measures of dry eye disease. The observed decrease in MBA following CAE exposure demonstrates that compensatory mechanisms are initiated during the CAE exposure, and that

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this compensation may provide the means to identify and characterize clinically relevant sub-populations of dry eye patients.

Future Research

The next step in this research is to better understand the link between ocular tear film surface compensation, ocular discomfort and other related signs and symptoms. Research needs to be done to create "best methods" for identifying the correct subpopulation of Dry Eye Patients to receive targeted therapy. There are a number of compensation tools through which patients compensate some of which are blink pattern, reflex tearing, narrowing of the palpeveral fissure and alteration in the constituents of the tear film. Within the tear film itself the different constituents and their availability may alter the tear film dynamics. To better understand these dynamics a baseline of subjects that display reproducible compensatory behavior must be identified. Therefore, as a follow on study it will be useful to re-enroll the same subjects that displayed compensation in the 4th chapter and re-expose them to the CAE to test if they have similar response. It is not well understood at this time if the subjects that displayed decreased MBA post-CAE will display this type of compensation on a reliable basis or just transiently. If this group does display a similar response then we can hypothesize that subgroup classification through the use of measuring compensation is a

meaningful method and would have great impact in terms of understanding

disease and it's treatment. In this follow on study special effort needs to be taken

to ensure the environment under which patients are evaluated emulates that of the

first study as much as possible.

Conclusion

This research established that the analysis of Tear Film stability within the natural

blink pattern is a viable, meaningful and accurate method for evaluating Dry Eye

severity. This was accomplished both using a manual methodology (VCMA) and

then subsequently using an automated video processing methodology (The OPI

2.0 System). Both methodologies were validated as useful, reliable tools for

evaluating Tear Film stability in the context of a natural blink pattern. The current

gold standard, TFBUT , was shown to have higher variability and a lack of

specificity in it's ability to evaluate Dry eye severity changes in subjects before

and after the CAE; the OPI 2.0 system was able to detect differences in Tear film

stability before and after subjects exposure to the CAE. It was demonstrated

through the use of the OPI 2.0 System that subpopulations of Dry Eye Patients

exist both in terms of compensation and response. In addition meaningful

correlations were shown between mean breakup area (MBA), staining and

discomfort.

In conclusion MBA is a meaningful endpoint in evaluating Dry Eye

Severity and the OPI 2.0 system is an accurate and precise tool to measure MBA.

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APPENDIX A

LIST OF PREVIOUSLY PUBLISHED WORK

CHAPTER 2 and CHAPTER 3 have been previously published. Please see the references below:

Abelson, R., Lane, K. J., Angjeli, E., Johnston, P., Ousler, G., & Montgomery, D. (2011). Measurement of ocular surface protection under natural blink conditions. *Clin Ophthalmol, 5*, 1349-1357.

Abelson, R., Lane, K. J., Rodriguez, J., Johnston, P., Angjeli, E., Ousler, G., & Montgomery, D. (2012). Validation and verification of the OPI 2.0 System. *Clin Ophthalmol, 6*, 613-622.

CHAPTER 4 has been accepted for publication to the Journal of Clinical Ophthalmology.

APPENDIX B

PERMISSION STATEMENT

All co-authors have granted permission for this research to be used for my dissertation.

APPENDIX C

SAMPLE SAS CODE

Independent Normal/Linear Model

```
proc genmod data=dataset;
class group;
model Staining = group / dist=Normal link=Id;
lsmeans group / diff;
run;
```

Independent Gamma Multiplicative Model

```
proc genmod data=dataset;
class group;
model IBI = group / dist=Gamma link=Log;
lsmeans group / diff;
run;
```

Correlated Normal/Linear Model

```
proc genmod data=dataset;
class subj group;
model Staining = group / dist=Normal link=Id;
lsmeans group / diff;
run;
```

Correlated Gamma Multiplicative Model

```
proc genmod data=dataset;
class subj group;
model IBI = group / dist=Gamma link=Log;
repeated subject = subj / type=exch;
lsmeans group / diff;
run;
```

APPENDIX D

SAMPLE SAS OUTPUT – DRY EYE VS NORMAL

```
Group    logIBI                                                    Cum.
Cum.
         Midpoint                                     Freq  Freq  Percent
Percent
                  |
DryEye    0.3     |*********************************     9     9    16.67
16.67
          0.9     |*********************************     9    18    16.67
33.33
          1.5     |*******************                   5    23     9.26
42.59
          2.1     |***********************               6    29    11.11
53.70
          2.7     |************                          3    32     5.56
59.26
          3.3     |********                              2    34     3.70
62.96
                  |
Normal    0.3     |                                      0    34     0.00
62.96
          0.9     |********                              2    36     3.70
66.67
          1.5     |**************************             7    43    12.96
79.63
          2.1     |***********************************    9    52    16.67
96.30
          2.7     |********                              2    54     3.70
100.00
          3.3     |                                      0    54     0.00
100.00
                  |
                  ----+---+---+---+---+---+---+---+---+
                      1   2   3   4   5   6   7   8   9

                            Frequency
```
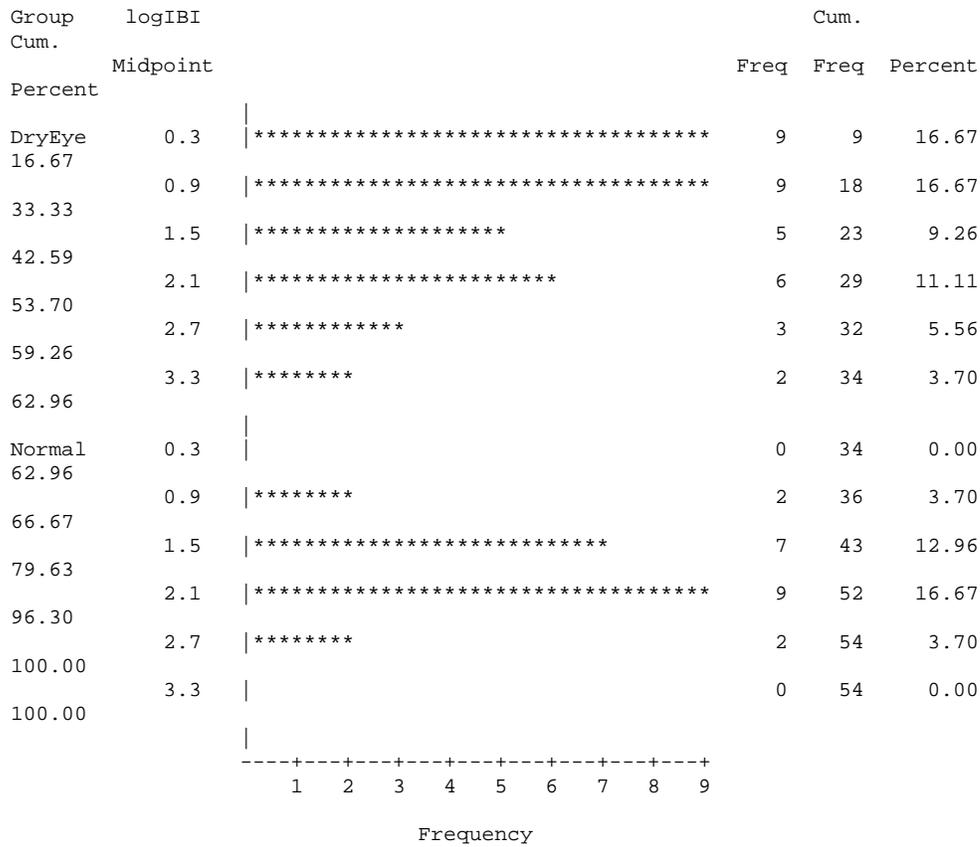
Sample: Eyes=54,
y=IBI, Dist=Gamma, Link=Log, AIC=298

group	Est	SE	Mean
Normal	1.92	0.17	6.82
DryEye	1.71	0.13	5.51

Parameter	group	Est	SE	Lo95	Hi95	Ratio	Pval
Intercept		1.71	0.13	4.28	7.10	5.51	<.001
Group_	Normal	0.21	0.21	0.82	1.87	1.24	0.315
Group_	DryEye	0.00	0.00	1.00	1.00	1.00	.
Scale		1.77	0.31	3.49	12.22	5.86	_
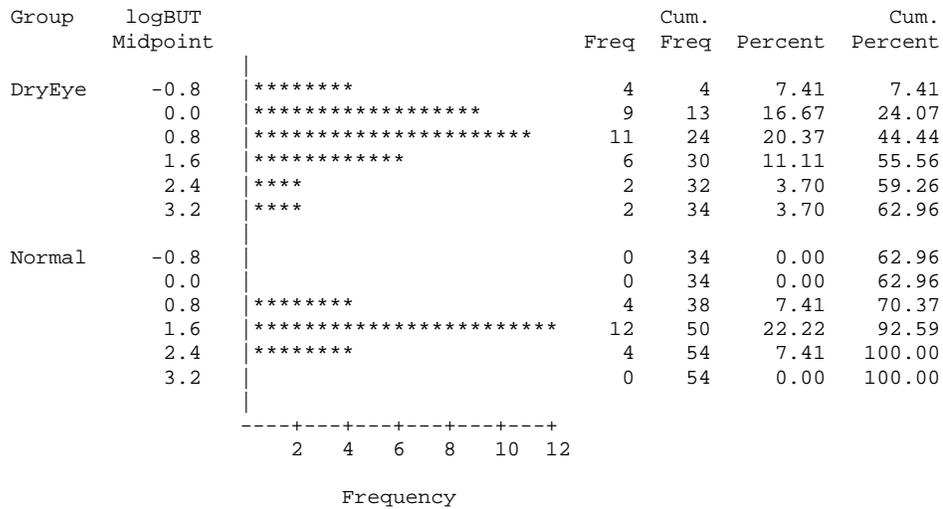
```
Group    logBUT                                       Cum.              Cum.
         Midpoint                            Freq  Freq  Percent   Percent
              |
DryEye   -0.8  |********                         4    4     7.41      7.41
          0.0  |******************               9   13    16.67     24.07
          0.8  |*********************            11   24    20.37     44.44
          1.6  |************                     6   30    11.11     55.56
          2.4  |****                             2   32     3.70     59.26
          3.2  |****                             2   34     3.70     62.96
              |
Normal   -0.8  |                                 0   34     0.00     62.96
          0.0  |                                 0   34     0.00     62.96
          0.8  |********                         4   38     7.41     70.37
          1.6  |***********************          12   50    22.22     92.59
          2.4  |********                         4   54     7.41    100.00
          3.2  |                                 0   54     0.00    100.00
              |
              ----+---+---+---+---+---+
                  2   4   6   8  10  12

                     Frequency



Sample: Eyes=54,
y=BUT, Dist=Gamma, Link=Log, AIC=271

group      Est       SE      Mean

Normal     1.68     0.19     5.39
DryEye     1.37     0.15     3.95



Parameter   group       Est       SE    Lo95     Hi95    Ratio     Pval

Intercept              1.37     0.15    2.97     5.25     3.95    <.001
Group_      Normal     0.31     0.24    0.85     2.18     1.36    0.193
Group_      DryEye     0.00     0.00    1.00     1.00     1.00      .
Scale                  1.39     0.24    2.69     7.11     4.03      _
```

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```
Group    logBUA                                              Cum.
Cum.
         Midpoint                                    Freq  Freq  Percent
Percent
                    |
DryEye   -5.25      |***************************       7     7    12.96
12.96
         -3.75      |                                  0     7     0.00
12.96
         -2.25      |****                              1     8     1.85
14.81
         -0.75      |******************                5    13     9.26
24.07
          0.75      |**********************            6    19    11.11
35.19
          2.25      |***********************************  9    28    16.67
51.85
          3.75      |**********************            6    34    11.11
62.96
                    |
Normal   -5.25      |                                  0    34     0.00
62.96
         -3.75      |                                  0    34     0.00
62.96
         -2.25      |***************                   4    38     7.41
70.37
         -0.75      |*******************               5    43     9.26
79.63
          0.75      |*************************          7    50    12.96
92.59
          2.25      |************                      3    53     5.56
98.15
          3.75      |****                              1    54     1.85
100.00
                    |
                    ----+---+---+---+---+---+---+---+---+
                        1   2   3   4   5   6   7   8   9

                              Frequency



Sample: Eyes=54,
y=BUA, Dist=Gamma, Link=Log, AIC=273

group       Est       SE       Mean

Normal     1.23      0.36      3.41
DryEye     2.36      0.28     10.56



Parameter    group      Est       SE      Lo95     Hi95    Ratio    Pval

Intercept              2.36      0.28     6.14    18.15    10.56    <.001
Group_       Normal   -1.13      0.45     0.13     0.79     0.32    0.013
Group_       DryEye    0.00      0.00     1.00     1.00     1.00     .
Scale                  0.39      0.06     1.33     1.69     1.47     _
```