The Role of the Meniscus in the Tear Film

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This PhD is in commemoration of my mother,

and dedicated to my lovely wife Marion

and my wonderful daughter Lynn.

SUMMARY

In the diagnosis of dry eye, an evaluation of tear fluid volume is an important parameter. The tear menisci hold approximately 75-90% of the overall tear fluid volume and serve as reservoirs, supplying tears to the pre-corneal tear film. The measurement of the anterior curvature radius of the tear meniscus (TMR) is an indicator of tear film volume and when it is performed non-invasively has been found to have good dry eye diagnostic accuracies. Optical coherence tomography and meniscometry are existing techniques that can measure TMR non-invasively. These techniques have not found wide application among clinicians, either because they are not commercially available or they are too expensive.

This PhD describes a series of experiments that investigated the development, evaluation and application of a new instrument for non-invasive tear meniscus measurement. From the results of these studies, it can be concluded:

A Portable Digital Meniscometer (PDM) was developed. This consists of an application tool for the iPod-touch, a slit-lamp holder for the iPod-touch and an image analysis software for TMR calculation. A simple iPod-touch or an iPhone mounted on a commercially available digital slit-lamp can be used to project a grid of black and white lines on the tear meniscus. Using the principal of reflective meniscometry, the radius of the lower tear meniscus can be non-invasively measured. This newly developed instrument is a simple, mobile and useful device for measuring tear meniscus radius, and therefore tear volume, and is suitable for use by clinicians.

The newly developed PDM was evaluated *in vitro* and *in vivo*. It produced accurate and reliable measurements and provided similar values for the tear meniscus radius, in human studies, to the existing video-meniscometer. PDM and OCT measurements of the TMR were significantly correlated. Since with the PDM no image calibration is needed, it seems to be a quick and non-invasive technique for evaluation of tear fluid quantity. The PDM appears to measure the radius of the central section of the tear meniscus

The PDM was able to non-invasively measure alterations in TMR and TMH along the lower lid. The flatter TMR and higher TMH at the nasal and temporal locations may be caused by the LIPCOF degree of the underlying conjunctiva. To avoid any interference by LIPCOF, it is recommended that TMR and TMH be measured along the lower lid margin below the pupil centre. Furthermore, the PDM was able to usefully detect changes in TMR following the instillation of artificial tears. The difference in residence time is likely to reflect the different viscosity and Newtonian properties of these drops. An overload with a large drop may result in initial increased blink rate. Blink rate at baseline is significantly related to dry eye symptoms.

CONTENTS

ABBREVATIONS

ACE	Angiotensin-converting-enzyme
ADDE	Aqueous deficient dry eye
ANOVA	Analyses of Variances
ATD	Aqueous tear deficiency
BAC	Benzalkonium chloride
BUT	Tear Break-up time
CCD	Charge-coupled device
CCH	Conjunctivochalasis
CCLRU	Cornea and Contact Lens Research Unit
CI	Confidence intervals
CL	Contact lens
CLDEQ	Contact Lens Dry Eye Questionnaire
CLEK	Collaborative Longitudinal Evaluation of Keratoconus
CLIDE	Contact lens induced dry eye
CMC	Carboxy-methylcellulose
cP	Centipoise
CR	Coefficient of repeatability
DED	Dry eye disease
DEQ	Dry eye questionnaire
DES	Dry eye syndrome
DET	Dry eye test
DEWS	International Dry Eye Workshop
EDE	Evaporative dry eye

FCT	Fluorescein clearance test
HEMA	Hydroxyethyl methacrylate
ICC	Intraclass correlation coefficient
Ig	Immunoglobulin
LIPCOF	Lid parallel conjunctival folds
LLT	Lipid layer thickness
LTM	Lower tear meniscus
LWE	Lid wiper epitheliopathy
MG	Meibomian Gland
MGD	Meibomian Gland Dysfunction
MUC	Mucin
NEI VFQ-25	National eye institute visual function questionnaire-25
NIBUT	Non invasive break-up time
OCI	Ocular comfort index
OCT	Optical coherence tomography
OSDI	Ocular surface disease index
PhD	Doctor of Philosophy
PCTF	Pre-corneal tear film
PDM	Portable digital meniscometer
PMMA	Polymethylmethacrylate
PRT	Phenol red thread test
RGP	Rigid gas permeable
SE	Standard error
SD	Standard deviation / Spectral Domain
SESOD	Subjective evaluation of symptom of dryness

SL	Slit lamp
SS	Swept Source
TD	Time Domain
TF	Tear film
TFLL	Tear film lipid layer
TFOS	Tear Film & Ocular Surface Society
TFV	Tear film volume
TM	Tear meniscus
TMA	Tear meniscus area
TMH	Tear meniscus height
TMR	Tear meniscus radius
TMV	Tear meniscus volume
TTR	Tear turnover rate
TVL	Tear volume loss
UTM	Upper tear meniscus
VM	Videomeniscometer

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CHAPTER 1: Introduction

Fifteen to thirty per cent of patients suffer from ocular-related symptoms such as stinging, burning, itching, light sensitivity and blurry vision, a group of symptoms often associated with dry eye syndrome (DES)[1-4]. Dry eye is a multi-factorial disease resulting in damage to the ocular surface and symptoms of discomfort, and principally due to an insufficient tear film[5]. This insufficiency is typically caused by an aqueous deficiency or increased evaporation of the tear film[5]. Additional exogenous causes can also induce dry eye even in normally asymptomatic patients. For example, contact lenses can cause a condition known as contact lens induced dry eye (CLIDE). Contact lens wearers are 12 times more likely than those who were clinically emmetropic (not requiring refractive correction per patient report) and five times more likely than spectacle-wearers to report dry eye symptoms[6]. About 50-75% of contact lens wearers report symptoms of dryness and ocular irritation[1, 6-10], and about 12% of contact lens patients discontinue lens wear within 5 years of starting due to these symptoms[11].

Many dry eye patients show increased staining, redness, excessive tear evaporation, decreased tear film stability, a hyperosmolar tear fluid and a reduced tear film volume[12-15]. However, correlations between dry eye symptoms and clinical signs are frequently poor[16-18]. Nevertheless, this lack of relation between signs and symptoms might be due to the technique of observation. For example, measurement of the tear meniscus height is used in many studies for tear volume assessment and in clinical practice it is mostly performed with a slit-lamp[19-23]. Yet, with slit-lamp examination the top of the meniscus cannot always be easily identified[24, 25] and therefore fluorescein is instilled, making the test invasive. In contrast, analysis of the tear

meniscus radius, while more difficult to do, is assumed to be better in predicting tear volume in a non-invasive way[26-29].

The tear meniscus radius can be evaluated by the use of an optical coherence tomographer[28, 30-33] or a meniscometer[26, 27, 34-37]. Although both instruments measure tear meniscus non-invasively, they have not found wide application among clinicians, either because they are not commercially available in all parts of the world or they are too expensive[14]. For example, one such meniscometer, invented by Prof. Dr. Anthony Bron, projects a defined grid of black and white lines onto the tear meniscus[27]. The meniscus acts as a concave mirror and the size of the reflected image is used to calculate the tear meniscus radius. However, worldwide, only three such video-meniscometers are in use.

This PhD project aims to (i) improve the evaluation of the tear meniscus for the clinician by developing an advanced observation device, (ii) investigate the relationship between the tear meniscus radius (TMR) and the tear meniscus height (TMH), as well as the effect of area of observation in normal and dry eye patients, and (iii) further explore the impact of tear supplements on the menisci.

CHAPTER 2: Literature Review

2.1. The Tear Film and Dry Eye

The tear film covers and lubricates the cornea, the bulbar conjunctiva and the palpebral conjunctiva, in order to maintain ocular surface health, to protect the ocular surface from mechanical forces during blinking, for nutrition of the cornea, and to enable a smooth layer over the cornea surface to obtain best optical quality of the otherwise optically irregular epithelial surface of the cornea. An insufficient tear film results in damage of the ocular surface and symptoms of dryness, burning, grittiness, scratchiness or soreness[38]. According to the international Dry Eye Workshop (DEWS), the disease is defined as:

Dry eye is as a multi-factorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with the potential for damage to the ocular surface[5].

2.1.1. Structure and Function of the Tear Film

The classic model describes the tear film as having three layers[39, 40]. The superficial lipid layer (about 0.1 μm in thickness) forms the anterior part of the tear film. Below this layer is an intermediate aqueous phase (approximately 7 μm), and an underlying mucous layer (0.02 to 0.05 μm), which is adherent to the microvilli of the corneal epithelium (Figure 2.1).

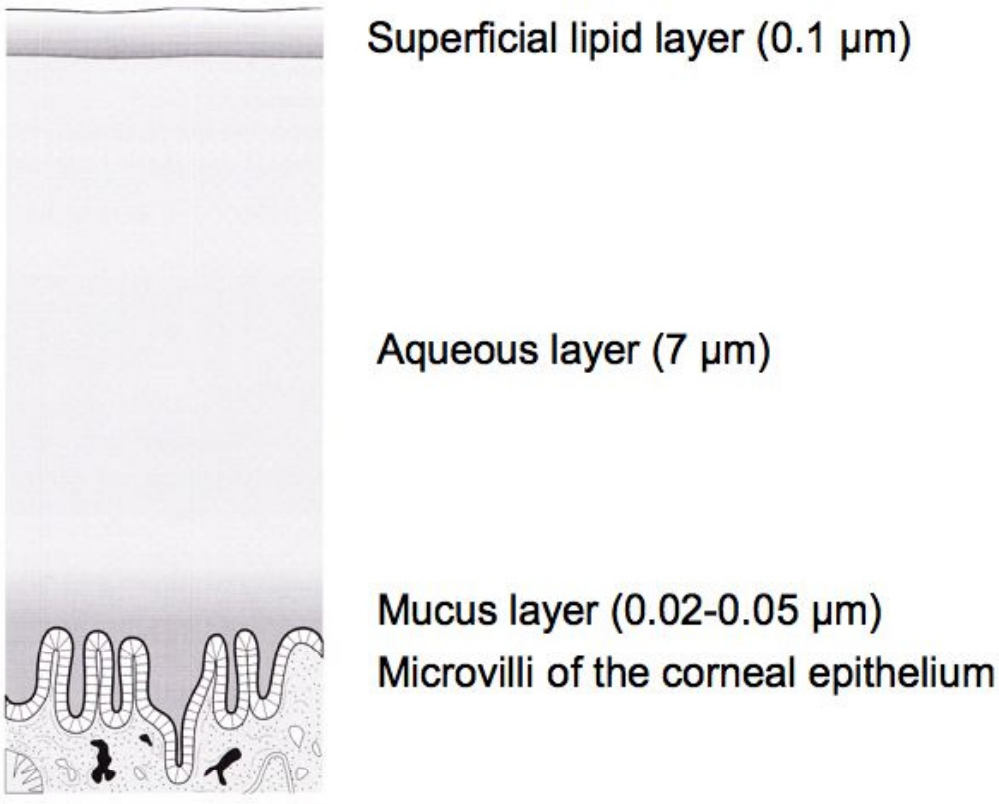


Figure 2.1: Classic three layer tear film model (from Patel and Blades, 2003)[39, 41].

Others have proposed a more complicated tear film model with six different layers[42]. Recent observations describe a tear film model in rats involving only two layers: mucins exist as a network distributed in the aqueous body forming a single mucin-aqueous layer, which is covered by an oil layer[43, 44]. Similarly, no evidence of a separate free-aqueous phase was found in mice, where the tear film was observed as an aqueous gel[45]. This is consistent with Dilly[46] who has shown that the concentration of dissolved mucins in the aqueous phase of humans decreases when moving towards the lipid layer.

A newer model of the tear film structure was published by Butovich et al. in 2008 (Figure 2.2)[47]. It consisted of a glycocalyx layer of secreted mucins attached to the corneal epithelium squamous cells, covered by an intermediate aqueous phase with soluble mucins, proteins and salts. The thickness of the aqueous/mucin phase is about 3-40 µm. A lipid layer of 13-100 nm thickness covers this phase, with inner polar lipids bordering the aqueous phase and an outer non-polar lipid phase bordering the air.

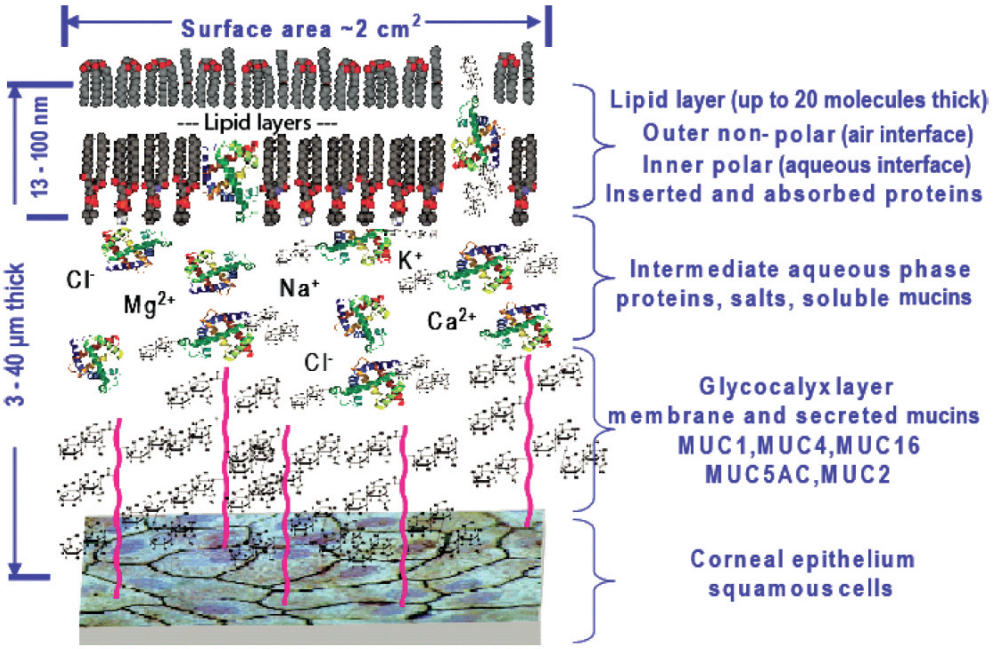


Figure 2.2: Diagram illustrating the tear film model (from Butovich et al.,2008)[47].

2.1.1.1. Lipids

The lipid layer is the outermost part of the tear film. It is produced by secretion from the Meibomian glands, which open onto the eyelid margins just anterior to the mucocutaneous junction. There are about 25 to 40 glands embedded in the upper lid and 20 to 30 glands in the lower lid[48, 49]. Delivery of oil to the lid margin reservoir in the tear meniscus is due, in part, to a steady secretory process and, in part, to the delivery of small aliquots with each blink, caused by the muscular action of the orbicularis muscle and the Riolan's muscles during the blink (Figure 2.3)[50-52].

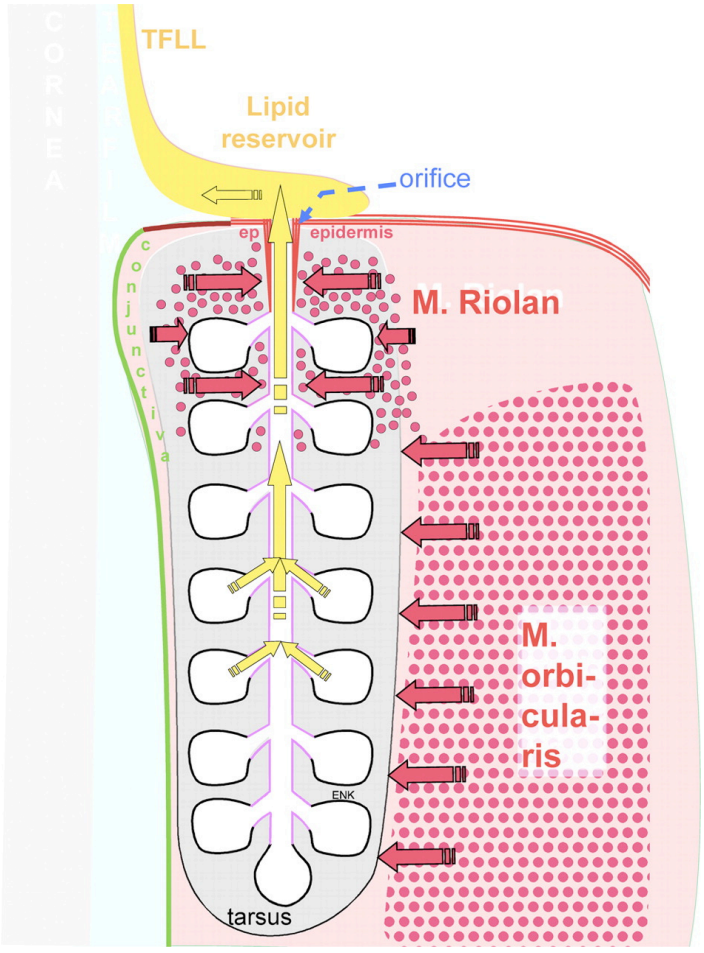


Figure 2.3: Diagram illustrating cross-sectional view of the lower lid with meibomian gland (from Knop et al., 2011)[52].

With the up-phase of each blink, the upper lid draws oil from the combined reservoir present between the apposed lids[50]. The surface tension gradient of the lipids on the aqueous phase causes a Marangoni flow of the aqueous tears from the tear menisci after each blink, leading to a thickening of the complete tear film (Figure 2.4)[53]. Yokoi et al.[54] demonstrated that the initial velocity of the tear film lipid layer (TFLL) spread after a blink increases steadily with increase of the radius of the tear meniscus. Therefore, they concluded that the rheological behaviour of the TFLL is influenced by aqueous tear film thickness over the cornea.

One important function of the lipid layer spread over the aqueous phase is to retard evaporation of the tear film. Furthermore, the hydrophobic meibomian oil prevents tear overspill at the lid margins[55]. Secretion of the meibomian glands consists of a mixture of non-polar lipids, polar lipids, free fatty acids, alcohols, hydrocarbons, wax esters, sterol esters and triglycerides[56]. The lipid layer is described as biphasic with a layer of polar and a layer of non-polar lipids[57]. The polar lipids form the inner layer at the aqueous-lipid interface and have a barrier function, the non-polar lipids are at the outer air-lipid interface and play an important role in the stability of the lipid layer[58]. More recent models take into account that there are also proteins inserted into the lipid layer which may have a significant role in binding the lipids to the aqueous[47].

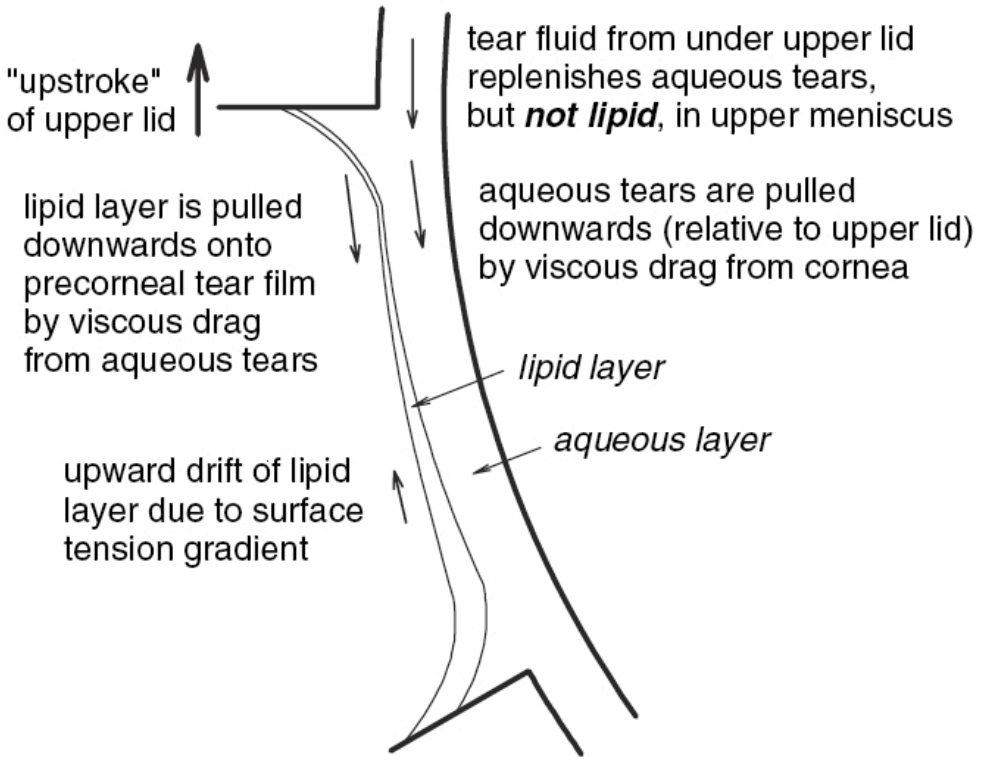


Figure 2.4: Diagram illustrating the distribution of the lipid layer following a blink (from King-Smith et al., 2004)[59].

The refractive index of the lipid layer varies, depending on the wavelength, from 1.53 at 400 nm to 1.46 at 750 nm[56]. The thickness of the lipid layer can be measured by various interferometric techniques and values vary in different studies between 13 and 100 nm[60, 61].

2.1.1.2. Aqueous Phase

The aqueous phase of tears forms the bulk of the lacrimal section. It is produced by the lacrimal gland (reflex tearing), located in the superior temporal angle of the orbit, and the accessory (Krause and Wolfring) lacrimal glands (basic tearing). The Krause glands are located in the fornices and the Wolfring glands are located in the supratarsal conjunctiva of the upper lid[62-65]. The aqueous phase contains electrolytes, proteins, enzymes, metabolites and epithelial cells. The major proteins are the immunoproteins lysozyme, lactoferrin, lipocalin, albumin, transferrin, as well as IgA, IgG and IgM[66]. Thus the proteins of the tear film play an important role in protecting the eye from infection. Electrolytes are actively secreted by the acinar and ductal epithelium of the lacrimal gland[53]. Furthermore, the conjunctiva can modify the tear film by absorbing or secreting electrolytes and water and by secreting proteins including mucins[67].

Normal tear pH lies within the range of 7.20 to 7.60, with the bicarbonate ions and proteins providing a buffering capacity[68, 69]. Osmolarity is the measure of solute concentration, defined as the number of osmoles of solute per litre of solution (osmol/L or Osm/L)[70]. A meta-analysis on human tear film osmolarity studies gives an average tear osmolarity of 302 ± 9.7 mOsmol/L in normal subjects, and an average of 326.9 ± 22.1 mOsmol/L in those with all types of keratoconjunctivitis sicca[71]. The ionic composition of normal human tears is listed in Table 2.1[72].

Ion	Concentration mmol · l^{-1}
Na^+	128.70
K^+	17.00
Ca^{2+}	0.32
Mg^+	0.35
HCO_3^-	12.40
Cl^-	141.30

Table 2.1: Ionic composition of normal human tears (from Ubels et al., 1994)[72].

The functions of the aqueous phase are a generalised wetting of the ocular surface, transport of debris, control of infectious agents, osmotic regulation and buffering against changes that would affect tear film homeostasis [66].

2.1.1.3. Mucins

The conjunctival and corneal epithelial surfaces are covered by mucins. Mucins are defined as glycoproteins that are heavily glycosylated, with 50–80% of their mass comprised of carbohydrate[73]. Mucins are present in the glycocalyx layer, as well as in solution within the tear fluid. Mucin is principally secreted by the goblet cells of the conjunctiva and the apical epithelial cells, but some mucin forms are also secreted by the acinar and ductal cells of the lacrimal gland[74]. The regional variation in goblet cell density is illustrated in Figure 2.5. Goblet cell density is greatest over the caruncle, plica semilunaris and inferior nasal palpebral conjunctiva. Open circles indicate the typical locations of accessory lacrimal glands[48, 75]. Currently, 19 mucin genes are known in humans and they can be subdivided into secreted mucins (MUC 2, 5AC, 5B, 6, 7, 9) and membrane associated mucins (MUC 1, 3A, 3B, 4, 16)[76].

Secreted mucins can further be classified as large gel-forming mucins, such as MUC5AC, and smaller, soluble mucins, such as MUC 2, 7, 9[77].

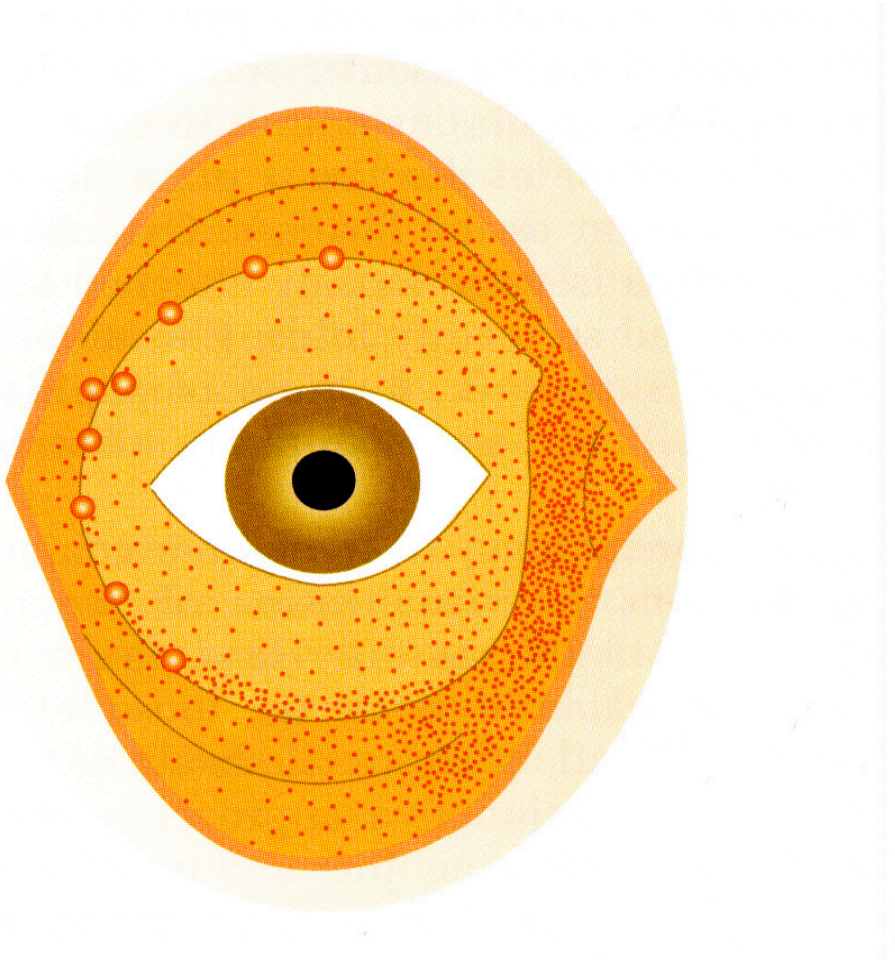


Figure 2.5: Regional variation in goblet cell density (from Efron, 2002)[48, 75].

The mucus layer forms a hydrophilic surface over the hydrophobic epithelium to facilitate an even spread and attachment of the aqueous component of the tear film[78]. The anchoring of a gelatinous layer of secreted mucin, so that a lubricated layer is present on all the surfaces gliding over each other during blinks and eye movements, is another major function of the mucins[53]. The large secreted mucins represent the

"janitorial service" that moves over the surface of the eye to wrap up and remove debris. The membrane-associated mucins form the glycocalyx, which provides a continuous barrier across the surface of the eye that prevents pathogen penetrance and has signalling capabilities that influence epithelial activity[73]. Furthermore, these mucins account for the fundamental non-Newtonian viscoelastic properties of the tear film, which allows the viscosity of the tears to change according to the shear rate of blinking[79]. The gel-forming MUC 5AC has been proposed to form the granular material overlying the glycocalyx, partly dissolved and mixed throughout the aqueous layer, along with other bactericidal proteins and fluids secreted by the lacrimal and accessory lacrimal glands[73].

2.1.2. Tear Production, Tear Flow Dynamics and Tear Drainage

The lacrimal system consists of a secretory system, which is responsible for tear production, and a drainage system to collect tears and regulate the outflow. In between the production and the drainage, the tear film needs to be transported over the ocular surface and to adhere to it. The total volume of tear fluid on the eye is estimated to be about 7µl[62] with a mean turnover rate ranging from 10.7% to 30% per minute[80, 81]. Normal tear film dynamics require a balance between production and elimination of tears from the eye (Figure 2.6).

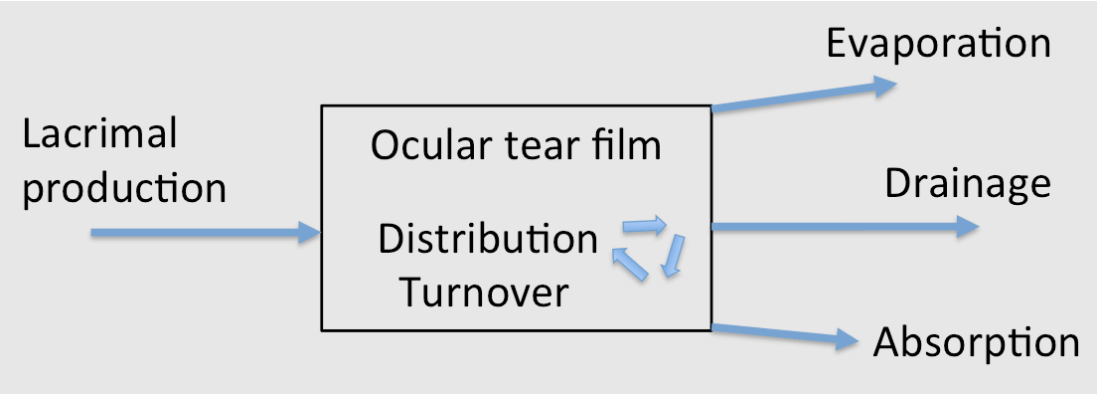


Figure 2.6: Input and output components of the tear system (from Tomlinson and Khanal, 2005)[82].

2.1.2.1. Tear Production

The secretory system includes the main lacrimal gland, which is located in the superior temporal angle of the orbit, and the accessory glands of Wolfring and Krause, which are found within the conjunctival fornices (Figure 2.7).

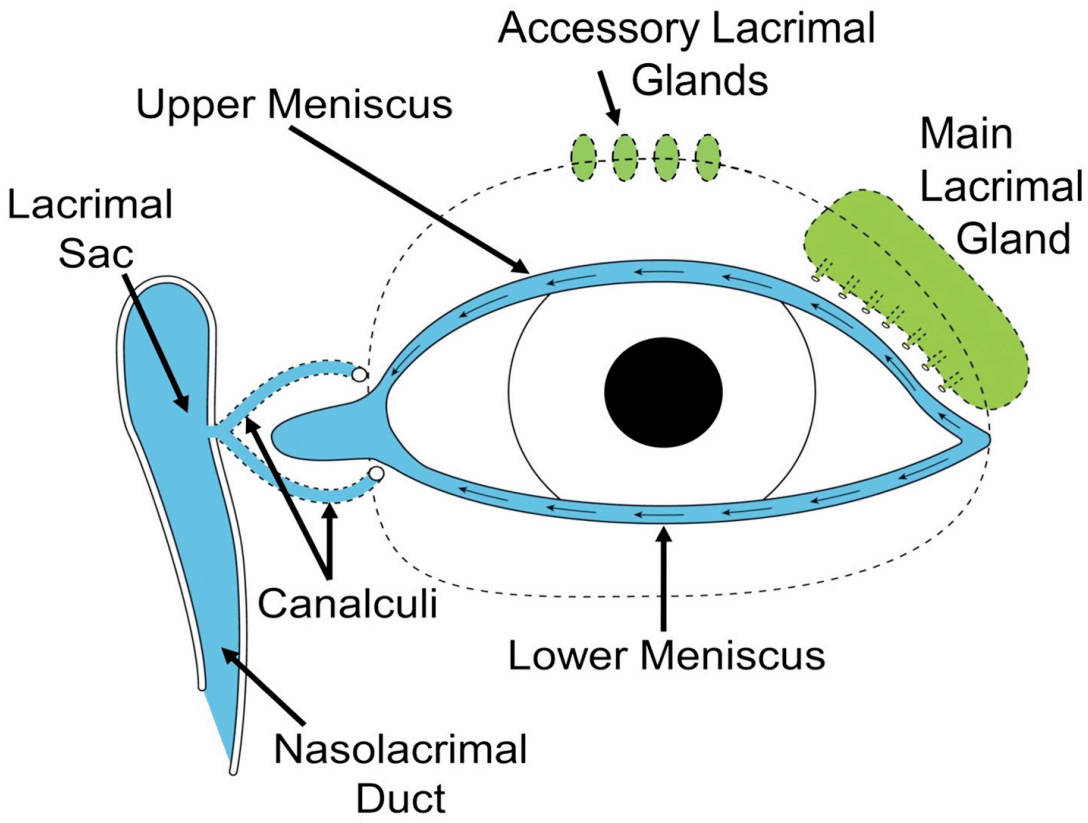


Figure 2.7: Diagram of production, tear flow and drainage of the lacrimal system (from Gaffney et al., 2010)[83].

The lacrimal gland consists of a larger orbital and a smaller palpebral division. At the palpebral lobe, between 6 and 12 ducts leave the gland and discharge into the conjunctival sac at the upper fornix[75]. The lacrimal gland is under the influence of parasympathetic and sympathetic nerves[75].

In tear production, a distinction can be made between basal tears, reflex tears and emotional tears[84]. The accessory glands are responsible for the basal tears with a production rate of between 0.19 and 1.2 µl/min[85, 86]. The functions of the basal tears are: compensating surface optical irregularities of the cornea, supplying the cornea

with oxygen and nutrition, providing anti-microbial factors and cleaning the ocular surface[84]. Reflex tears are reactions to stimuli from the environment, like coldness, mechanical irritation, injuries, odours or chemical agents[84]. With stimulation, tear production can increase up to 100 µl/min[85]. Emotional tears can be defined as a complex secretomotor phenomenon characterised by the shedding of tears from the lacrimal apparatus, without any irritation of the ocular structures, and often accompanied by alterations in the muscles of facial expression, vocalisations, and sobbing[87]. Emotional tear production rate can raise up to 400 µl/min[84].

Tear production is regulated by a reflex loop. Stimulation of nerves at the ocular surface sends impulses to the brain via the fifth cranial nerve, which generates a reflex response via nerves passing to the lacrimal glands (Figure 2.8)[88].

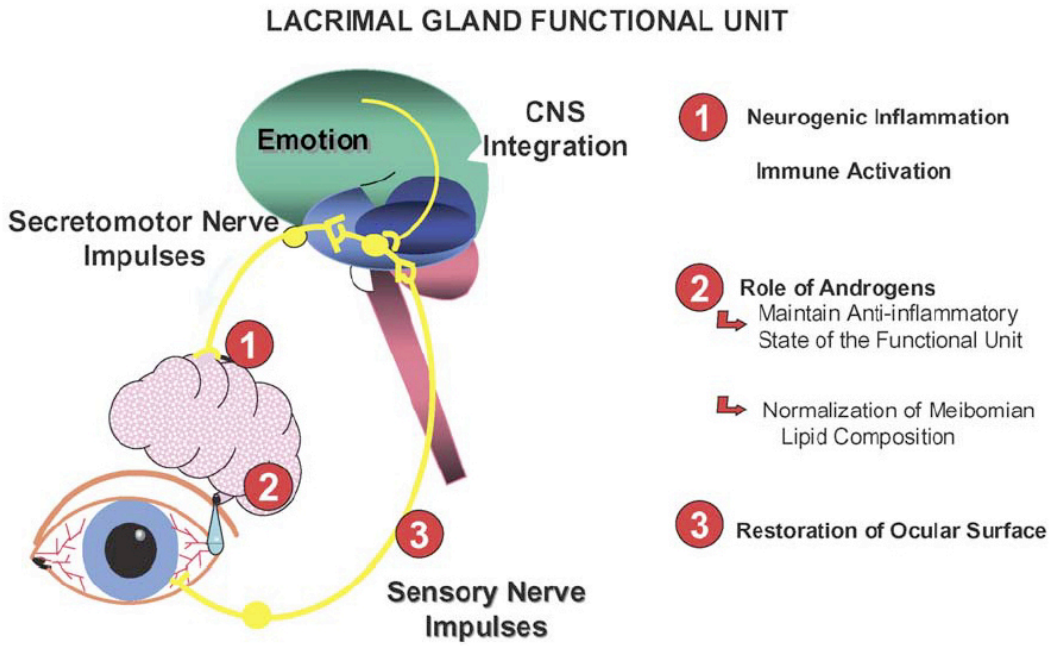


Figure 2.8: Schematic diagram of the lacrimal functional unit (from Stern et al. 2004)[88].

Any irritation of the ocular surface, e.g. low humidity, excessive evaporation or contact lens wear, may result in chronic afferent stimulation which results in increased lacrimal secretion[79, 89]. On the other hand, contact lens wear results in a decreased corneal sensitivity. Depending on the type of contact lens worn (PMMA, RGP or soft contact lenses) and the duration of lens wear, a different amount of depression in corneal sensitivity has been reported[90-93]. So this leads to the hypothesis that the different stimulus of the various contact lens types may correlate with a decrease in tear film production and therefore in a change of the tear meniscus.

Beside the secretion of proteins, electrolytes and water from the main gland and accessory glands, the epithelial cells of the cornea and conjunctiva also contribute to the tear film[67]. While the cornea produces a small proportion of the aqueous layer and mucins in the glycocalyx, the conjunctiva secretes substantial electrolytes and water into the aqueous layer and mucins into the mucous layer[67, 94].

2.1.2.2. Tear Flow Dynamics

Once tears are produced, they flow from the glands into the conjunctival sac and from there into the tear menisci[83]. The negative hydrostatic pressure within the meniscus seems to cause the flux of the fluid from the fornical sac into the meniscus[95]. Blinking then spreads the tears as a film from the menisci over the eye. In normal subjects, blinking occurs with a frequency of between 10 and 30 blinks per minute and refreshes the tear film[96]. During the closing phase of the blink, the lipid layer is compressed into the Kessing's space. On the opening phase of a blink, the lipids are spread over the eye causing a reduction in surface tension gradient[82, 97].

This allows the aqueous tears to follow the lipids and to be pulled out from the upper and lower tear menisci[83]. Once the blink has stopped and the eye is open, there is a flux from the tear film into the meniscus during the inter-blink period that is predominantly driven by capillary forces[83]. The low-pressure gradient in the meniscus causes tangential flow out of the neighbouring tear film into the meniscus[95, 98]. This causes the formation of a black line near the meniscus representing a thinning of the tear film close to the meniscus[95, 99].

Blinking not only spreads the tear film over the eye, it also pushes the tears along the menisci towards the puncta. While the upper lid mostly moves in the vertical plane during lid closure, the lower lid movement is mainly horizontal. In a study by Harrison et al.[100] it was observed that tear flow along the lid margins towards the punctum was much slower along the upper lid compared to the lower lid. After the instillation of fluorescein under the temporal upper lid it took 3 seconds for the fluorescein to spread laterally along the lower lid, but on the upper lid, even after 35 seconds, it was still only 2/3 of the way across the tear meniscus.

2.1.2.3. Tear Drainage

Three different routes are involved in elimination of tear fluid from the eye: evaporation, absorption of the fluid from the ocular tissue, and outflow of the fluid through the puncta.

During lid closure, the upper and lower puncta press on each other so that no fluid can flow out, while, at the same time, the action expels tears in the lacrimal sac, which produces a negative pressure in the sac[101]. When the lids open, tear fluid is

sucked through the puncta by capillary attraction into the canaliculus and on into the lacrimal sac[101]. After the blink, when the orbicularis relaxes, the sac collapses and this drives the accumulated tears into the nasolacrimal duct[66].

As the tear film in the open eye borders to the ambient air, a certain amount of fluid is lost by evaporation. The lipid layer of the tear film is very effective in preventing most of the evaporation from the ocular surface and reduces the evaporation of water by about 80-90% in the normal eye[82, 102]. Depending on the measuring techniques, the values for tear evaporation rate varies from 0.04 ± 0.01 ml/min[103] to 0.16 ± 0.04 ml/min[104] in normals, and between 0.15 ± 0.11 ml/min[82] and 0.58 ± 0.23 ml/min[98] in dry eye patients.

Tears might also be eliminated from the eye by absorption into the tissues of the ocular surface, since lipophilic substances of the tear fluid were absorbed in the nasolacrimal ducts in rabbits[105]. However, evidence for absorption of water by the corneal or conjunctival epithelium is lacking[53].

2.1.3. Volume and Distribution of the Tear Fluid

The tear fluid on the eye is present in three sections: at the exposed area between the lids covering the cornea and sclera, in the tear menisci at the lid margins and in the conjunctival sac of the upper and lower lid (Figure 2.9).

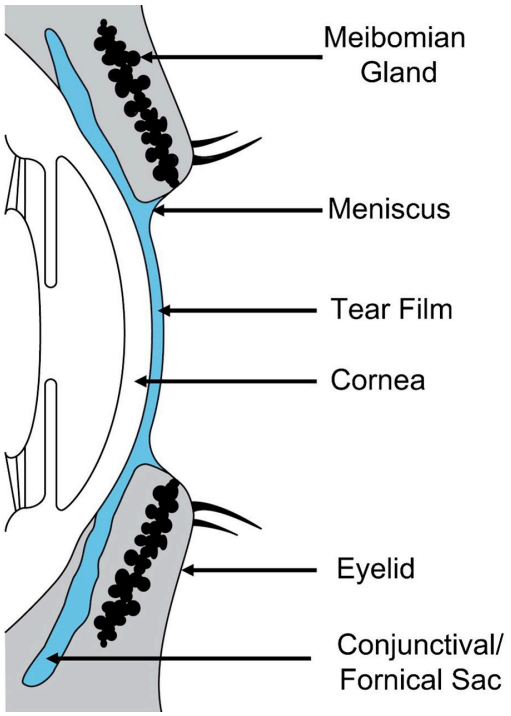


Figure 2.9: Sagittal view of the eye to show tear distribution (from Gaffney et al., 2010)[83].

The interpalpebral exposed area can be roughly calculated by the relationship between palpebral height and area: Area (cm^2) = 0.28 x (palpepral height in mm) – 0.44[106]. This formular is the result of a study by Rolando and Refojo[106] where they photographically measured the exposed area between the lids in cm^2 and found that it was sufficient to measure only the palpebral aperture in order to determine the area

of the exposed surface. With the assumption of a tear film thickness between 3 and 10 μm and an exposed area of 2 cm^2, the calculated volume of the preocular tear film is 0.6 – 2.0 μl, with a mean of about 1.0 μl[53]. The volume of tears lying under the lids is still unknown, or whether this should be included as part of the tear film[53]. There seems to be evidence that the exposed and the under-lid compartments of tear film are connected to each other[29, 59]. The mean under-lid volume has been calculated to be 5-6 μl[53].

Using a mean value of 0.365 mm for the tear meniscus radius of curvature and a total length of about 50 mm for the upper and lower meniscus, the normal meniscus volume is about 2.9 μl[107]. Thus, the calculated volume of tear meniscus is dependent on the measured curvature of the meniscus and the assumption that the meniscus is regular along the lid. All these parameters will result in different calculations of tear meniscus volume (see Section 2.3.). The more precise the evaluation of tear meniscus curvature and distribution, the more exact will be the volume of tear fluid calculated.

2.1.4. Definition of Dry Eye Syndrome

Dry eye syndrome (DES) is defined as a multi-factorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with the potential for damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface[5]. Although the distinctions of aqueous-deficient dry eye and evaporative dry

eye were removed from the older definition, they are still retained in the aetiopathogenic classification of dry eye (Figure 2.10)[5].

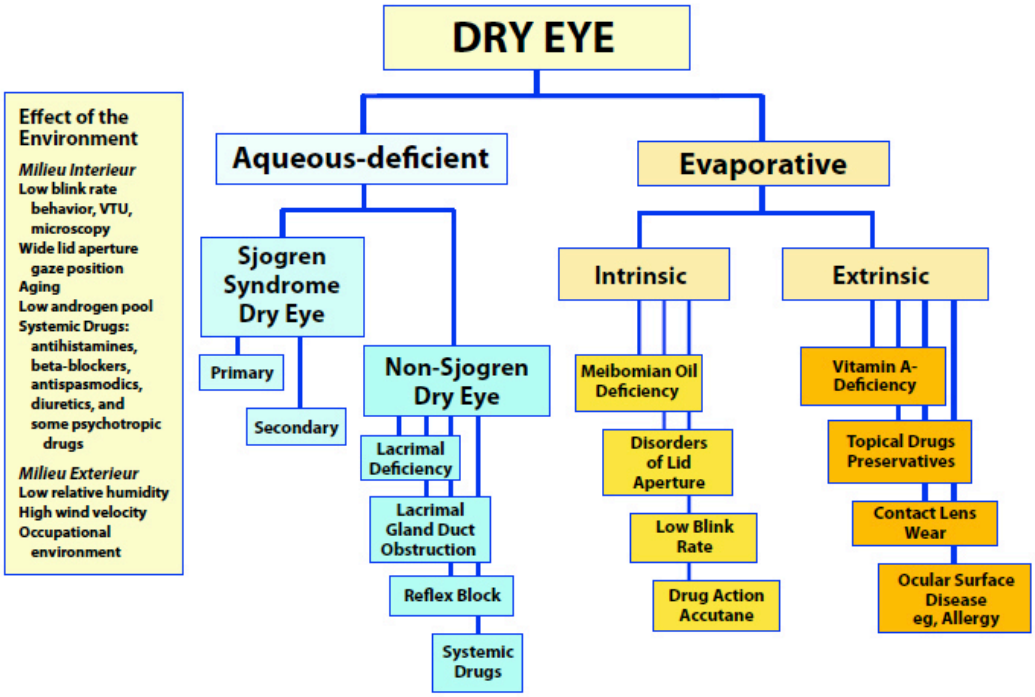


Figure 2.10: Major aetiological causes of dry eye (from the Dry Eye Workshop, 2007)[5].

Dry eye is grouped into evaporative dry eye (EDE) and aqueous-deficient dry eye (ADDE). In EDE, the lacrimal secretory function is normal, but there is excessive water loss from the ocular surface. In ADDE, the tear evaporation rate from the ocular surface is normal, and the dryness is due to a reduced lacrimal tear secretion. EDE can be further subdivided according to intrinsic or extrinsic causes. The most frequent intrinsic cause of EDE is meibomian gland dysfunction (MGD), leading to a

deficient tear film lipid layer[50, 108, 109]. The International Workshop on Meibomian Gland Dysfunction defined:

Meibomian gland dysfunction (MGD) is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. It may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease[110].

ADDE can also be subdivided as Sjögren's or Non-Sjögren's Syndrome Dry Eye. Sjögren's Syndrome is an auto-immune disease in which immune cells attack and destroy the exocrine glands that produce tears and saliva, leading to symptoms of dry eye, mouth and lips. Depending on the definition of dry eye and the various ages in epidemiological studies, the prevalence of dry eye ranges from about 5% to over 35%[111-114].

ADDE and EDE can also occur together, and a correct diagnosis of the different subtypes of dry eye is challenging, but important for an effective and successful treatment of dry eye. However, a retrospective study by Lemp at al.[115] found in a group of 224 patient with dry eye, 35% to have EDE, 10% with ADDE, 25% with a mixture of MGD/ADDE and the remaining 29% were not found to have clear evidence of ADDE or EDE.

2.1.5. Factors affecting Dry Eye and Tear Meniscus

2.1.5.1. Gender

Women seem to be more affected by dry eye than men[112, 116, 117], although there are some studies that disagree[118, 119]. In an older American population (over 65 years), Schein et al. reported no association between gender and dry eye prevalence[118]. Tong et al. even found a higher prevalence in men than in women[119]. Their study was with Malays and they argued that the gender effects not only may differ in the older population, but also in Malays compared to Asians or Caucasians.

It is believed that the higher incidence of dry eye in women is due to hormonal influence or hormone replacement therapy[120-123], but a long-term incidence study by Moss et al.[117] found no association of dry eye incidence with a history of hormone replacement therapy. This contrast may be explained by the older population (63 ± 10 years) used in the study. Androgen levels decrease with ageing in both men and women[124]. Androgens regulate the lacrimal gland and appear to account for many of the gender-related differences that exist in the anatomy, molecular biology, physiology and immunology of this tissue[125]. With a deficit in androgens, lacrimal gland dysfunction, decreased tear secretion and aqueous-deficient dry eye seem to be more likely[125]. Furthermore, androgens appear to regulate meibomian gland function, improve the quality and/or quantity of lipids produced by this tissue and promote the formation of the tear film's lipid layer[125]. Krentzer et al. found that patients taking an anti-androgen treatment had a significant decrease in tear film break-up time and quality of meibomian gland secretions, and they hypothesised that

androgen deficiency is a critical aetiological factor in the pathogenesis of meibomian gland dysfunction and evaporative dry eye[126].

On the other hand, the role of oestrogens in the anterior eye is controversial. A study of over 25,000 post-menopausal women demonstrated that women using oestrogen replacement therapy have a significantly higher prevalence of severe dry eye symptoms and clinically diagnosed dry eye syndrome[122]. Other investigators have found no demonstrable influence of oestrogens on various aspects of the normal or autoimmune lacrimal gland or the tear film[125]. In a study by Tomlinson et al., no effect on tear physiology was found for serum hormone changes induced by oral contraceptive use or by normal cyclic variations in healthy young females[127].

Albietz et al.[113] developed a sub-type based dry eye diagnostic protocol to determine the prevalence of the dry eye sub-types, namely lipid anomaly dry eye, aqueous tear deficiency, primary mucin anomalies, allergic/toxic dry eye and primary epitheliopathies, and lid surfacing/blinking anomalies. Aqueous tear deficiency, diagnosed by phenol red threat test and tear meniscus height evaluation, was the only sub-type with a significant gender prevalence difference, being more prevalent in women. Also, using a graticule at the slit-lamp to determine TMH, Patel et al.[128] reported no significant difference in the trends relating tear meniscus height with age between genders. However, in this study the TMH in females remained fairly stable between 0 to 40 years, but increased between 41 to 80 years. In contrast, in males a major increase in TMH was found between 21 to 60 years and thereafter remained stable.

In summary, ADDE seems to be more prevalent in women, with an increasing age-

related risk in women and men. Women tend to receive a diagnosis at a younger age than men, with the highest rates of dry eye disease in women aged 75 to 79 years and men aged 80 to 84 years[129]. In EDE, there seems to be no gender factor, but rather an age-related difference. Thus, the tear meniscus, as an indicator of tear volume and aqueous deficient dry eye, may be influenced by gender.

2.1.5.2. Age

Symptoms of dry eye are more prevalent in an older population[86, 112, 116, 117]. There is also an increase in the prevalence of meibomian gland anomalies[130] and ADDE[112, 118, 131]. The prevalence of dry eye ranges from 5.5%[112] to over 35%[114], depending on the age of the subjects, but also on the definition of dry eye in the study. Albietz found a dry eye prevalence of 18.1% in subjects of 40 years or older, compared with 7.3% in those <40 years[113]. In a study by Yazdani et al., patients aged ≥65 years were about 4 times as likely as those aged ≤65 years to be diagnosed with keratoconjunctivitis sicca or tear film insufficiency[129]. Using the Schirmer test or the phenol red thread test, the tear volume seems to decrease with advancing age[86, 132]. In contrast, Patel et al. found a gradual increase in TMH with advancing age[128]. They argued that this may be attributed to the fact that the Schirmer and phenol red threat tests used in the other studies are more invasive than TMH measurement, which suggests that the different tests are measuring different aspects of the tear film. Furthermore, they observed a smaller puncta diameter with increasing age and concluded that any effects on tear volume by a thinning lipid layer are outweighed by changes in the puncta[128]. Qiu et al.[133] found TMH values measured with OCT were negatively correlated with age in healthy Chinese subjects, while they could not find a correlation with age in dry eye subjects. Also, using OCT, Cui et al.[134] confirmed

the finding of a decreasing TMH with age in a healthy group.

2.1.5.3. Ethnicity

In a recently published questionnaire-based study by Tong et al.[119] the dry eye prevalence in a Malaysian population was found to be 6.5%, and to be higher in men compared to women, although the prevalence in men decreased in those over 60 years old. These findings are contrary to similar questionnaire-based studies in the US and in Indonesia, with reported prevalence rates of 15%[135] and 27.5%[136], respectively. A higher prevalence of dry eye in elderly Asians compared to Caucasians was reported by Lin et al.[114]. Shen et al.[28, 137] noted that for Chinese subjects the lower tear meniscus height was greater than the upper tear meniscus, which differs from the result of an US study[32] where the lower and upper tear menisci were similar. They attributed the differences to the narrow apertures and the tight eyelids in Chinese eyes. In conclusion, the prevalence of dry eye is not only influenced by age and gender, but also by the ethnic background of the study population. This may be caused by differences in climate or other environmental conditions, food pattern, anatomical variation, quality of life, or the prevalence of other dry eye associated diseases.

2.1.5.4. Systemic Diseases

Dry eye is associated with several other systemic diseases. A strong association between arthritis, allergies, diabetes mellitus, thyroid disease and dry eye has been noted in different studies[112, 116, 117, 119, 138-142]. Other conditions, such as connective tissue disease, radiation therapy, stem cell transplantation, vitamin A deficiency,

hepatitis C infection, androgen deficiency, blepharitis, HIV infections, sarcoidosis, ovarian dysfunction and pterygium may also be risk factors for dry eye[111, 136].

There are few papers in the literature about the influence of different diseases on tear meniscus height. Thus is because subjects with different diseases are excluded in most of the studies on tear meniscus height. However, in one study by Francis et al.[143], a significantly greater tear meniscus height was found in patients with primary acquired nasolacrimal duct obstruction and with functional nasolacrimal duct obstruction.

2.1.5.5. Drugs

Several classes of drugs, including anti-histamines, diuretics, anti-depressants, anti-anxiety drugs, beta-blockers, systemic chemotherapy, selective serotonin re-uptake inhibitors, and oral steroids are associated with an increased dry eye risk, whereas other drugs like ACE inhibitors are associated with a decreased risk[111, 117]. All drugs blocking the parasympathetic nervous system may result in a reduced tear film production and an aqueous deficient dry eye[144]. These are, for example, anti-histamines, anti-depressants, anti-anxiety drugs, cycloplegica or anaesthetics. Several drugs blocking the sympathetic nervous system like beta-blockers or anti-hypertensives may also lead to a reduction in tear film production[144]. The use of isotretinoin, which is a systemic vitamin-A derivate used to cure acne, causes signs and symptoms of dry eye, probably by reducing meibomian gland function[145]. Preservative agents in eye drops like benzalkonium chloride (BAC) have been shown to cause tear film instability and loss of goblet cells[146].

2.1.5.6. Diet

Essential fatty acids are necessary for complete health and they are assumed to play an important role in dry eye[147]. A large epidemiological study involving 39,876 women in the USA found that higher intake of omega-3 fatty acids in the diet was associated with a decreased incidence of dry eye syndrome. Women who consumed 5 or more servings of tuna per week were at a 68% reduced risk of dry eye syndrome, compared to women who had one serving per week[148]. They also found that the higher the dietary ratio of omega-3 to omega-6 essential fatty acids, the lower the likelihood of dry eye. In a typical western diet, 20-25 times more omega-6 (i.e. hamburgers, pizza, ice cream, potato chips) than omega-3 (cold water-fish such as salmon, mackerel, tuna and sardines) acids are consumed[149]. A significant increase in tear production, as defined by TMH, after six month of orally administered omega-6 fatty acids in contact lens associated dry eye was observed in a study by Kokke et al. [150]. As a result of this, some researchers suggest using omega-3 and others using omega-6 or a combination of both[151]. The questions of what combination, dose or length of treatment is best for treating dry eye remain unanswered[152].

Vitamin A deficiency has many ocular manifestations, including night blindness, xerophthalmia, and loss of vision[153]. Vitamin A deficiency reduces the number of goblet cells which produce the majority of the mucin of the tear film[41]. Dry eye is frequently accompanied by a loss of conjunctival goblet cells[154].

2.1.5.7. Diurnal Variation

Tear meniscus parameters are dynamic and there are fluctuations during the day. Tear menisci are significantly elevated at eye opening, after overnight sleep, compared to the values before sleep[137, 155]. A significant reduction in the central TMH occurs over the course of the day, with no significant difference between dry eye and non-dry eye patients[156]. TMH peaks upon eye opening and decreases during the day until a minimum is reached before sleeping (Figure 2.11).

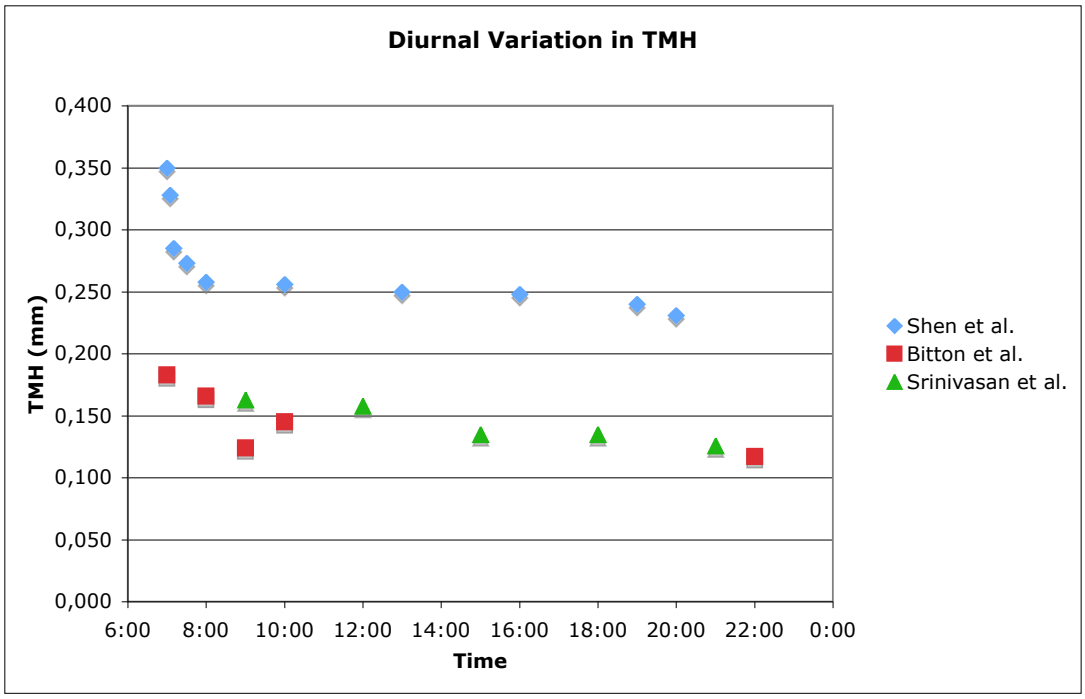


Figure 2.11: Diurnal variations in tear meniscus height as described in different studies.

2.1.5.8. Blinking

Blinking is an important aspect in the secretion, spreading, evaporation and drainage of tears[157-159]. Since a reduced blink rate lengthens the period of open eye, the ocular surface will be exposed much longer than in a normal blink rate. This may cause a 'drying' of the ocular surface[160]. Indeed, Tsubota and Nakamori showed that tear evaporation increased proportionally with the ocular surface area and that tear evaporation was dependent on blink rate[159]. The blink rate and maximum blink interval were significantly different in dry eye patients compared with healthy volunteers in a study by Nakamori et al.[161]. In addition they showed that the use of video display terminals was associated with a decreased maximum blink interval and dry eye symptoms. The blink rate therefore is influenced by various factors, such as ocular irritation, precorneal tear film condition, visual demands, or environmental conditions[159, 161, 162].

Besides having an effect on evaporation, the spreading, secretion and drainage of the tear film on the eye, and therefore the tear meniscus, is also influenced by blinking. The TMH of the inferior and superior menisci initially swell by the same amount following a blink, but thereafter the profile rapidly becomes eccentric, with the radius of the superior meniscus exceeding that of the inferior[31,163]. However, even with a lower tear meniscus volume, a stable tear film can be deposited by the superior meniscus alone, without contribution from the inferior meniscus after a partial blink[100]. Blinking is also important for the distribution of instilled artificial tears. Palakuru et al.[164] showed that an increase in blink output helps to restore balance when the tear system is overloaded with instilled tears. They also confirmed

that at the end of the eye-opening period, the inferior tear meniscus volume increased significantly.

Recent studies suggest that not only the frequency, but also the completeness of blink, may have an effect on dry eye symptoms[165, 166].

2.1.5.9. Contact Lens Wear

About 50-75% of contact lens wearers reporting symptoms of dryness and ocular irritation[1, 7-9] and about 12% of contact lens patient discontinue lens wear within 5 years due to these symptoms[11].

Although in contact lens wearers the TMH values were not statistically different from those of the control group while wearing lenses, Miller et al. showed a tendency for these values to be lower in daily hydrogel and silicone hydrogel lens wearers, but not in gas-permeable lens wearers[20]. In their study they used a graticule to measure TMH and wearers that were adapted to the lenses with a successful history of lens wear. TMH was also unaffected by 18 months daily and continuous wear of silicone hydrogel lenses in a study by Santodomingo-Rubido et al.[167]. Comparing non-wearers and daily soft lens wearers, Guillon et al. also could not find a difference in TMH measured with a variable slit-lamp beam height. As they compared asymptomatic and symptomatic daily soft lens wearers, subdivided by the McMonnies questionnaire, they reported a significantly higher TMH for the symptomatic lens wearing subjects, which was attributed to reflex tearing[168]. In contrast, in a study by Glasson et al., TMH was significantly reduced in intolerant wearers, and formulae including NIBUT, symptoms and TMH measurement were

found to be able to predict contact lens intolerance with high sensitivity and specificity[169]. Using optical coherence tomography (OCT), Wang et al. could not detect a significant difference in TMH, tear meniscus radius (TMR) and tear meniscus area (TMA) between wearers of silicone hydrogel lenses made of balafilcon A and galyfilcon A, but they found an increase in TMH, TMR and TMA on insertion of contact lenses, which returned to baseline after 20 minutes[33, 170]. Wearing hydrogel lenses (vifilcon A) for a 6 hour period did not show any significant difference in TMH or TMA compared to the baseline values[171]. However, long-term wear (6 years on average) of hydrogel contact lenses (etafilcon A) could induce decreased tear volume[172]. Also, using OCT, there was a significant difference in calculated tear volume between dry eye symptomatic wearers and asymptomatic wearers of hydrogel daily disposable lenses (etafilcon A) with lower tear volumes in the symptomatic group[173]. In a later study by the same author with the same lens type, it was shown that tear volume decreased gradually during daily lens wear and that it contributed to the ocular comfort in both symptomatic and asymptomatic wearers[174]. In a group of overnight wearers of soft contact lenses, Tao et al. reported that tear meniscus volumes in CL wearers were less than those in controls at eye opening. The TMH in rigid gas permeable (RGP) lens wearers was found to be lower than in non-lens wearers (0.20 ± 0.08 vs. 0.28 ± 0.10 mm)[175].

In summary, tear meniscus height increases after the insertion of a contact lens due to reflex tearing. Depending on the different intensity and duration of the corneal stimulus caused by the contact lens material and the duration of lens wear, the sensitivity of the cornea may decrease leading to a reduced tear production and, therefore, volume. This may result in symptoms of dryness. The differences in

findings between some studies could be explained by an inadequate technique used to detect small changes in tear meniscus. However, based on the findings of studies by Glasson et al.[169] and Chen et al.[174], the TFOS International Workshop on Contact Lens Discomfort concluded that lower tear meniscus volume has a weak, but significant, relationship with discomfort in CL wear[176].

2.1.5.10. Eye Drops

The instillation of an increasing volume of balanced salt solution causes the radius of the lower tear meniscus to increase linearly[29]. Furthermore, central tear film thickness, TMH, TMR and TMA of the inferior and superior menisci increases as isotonic sodium chloride solution is instilled[177]. For tear film thickness, the elevation remains for 5 minutes and all other variables return to baseline after 20 minutes[177]. In their study, Wang et al. used tear drops with different viscosities, ranging from 1 cP to 70 cP, and found an increase in tear film thickness and inferior meniscus height, radius and area with the more viscous drop. The more viscous the drops were, the higher the increase in tear film parameters. No correlation was found with the dimensions of the superior tear meniscus, and with all drops the effect was gone 20 minutes after instillation[177]. Palakuru et al.[164] used 1.0% carboxymethylcellulose and observed an increased tear volume with a major increase in the inferior tear meniscus volume at five minutes post-instillation.

To summarise, the tear meniscus parameters increase when either the volume or the viscosity of the on eye fluid is raised.

2.1.5.11. Environmental

Low humidity environments, such as air-conditioned and office environments, are known to cause irritative complaints of dryness[178-180]. Low relative humidity, high temperature and reduced atmospheric pressure increases the water evaporation from the preocular tear film[178]. Working with a visual display unit may destabilise the tear film by encouraging a lower eye blink frequency and a larger exposed ocular surface area[161, 178]. Smoking seems to damage the lipid layer of the tear film and causes changes in tear proteins, which correlates with an increase in dry-eye-related subjective symptoms[181, 182].

2.2. Evaluation of the Tear Meniscus

The tear menisci along the superior and inferior lid margins represents 75 to 90% of the tear film volume at the ocular surface[183], although a lower estimate of 27% has been made[83]. The shape of the menisci is described to be roughly wedge-shaped in sagittal section, with a concave anterior surface, and posterior and peripheral surfaces that bathe and moisten the hydrophilic mucosae of the cornea and bulbar conjunctiva or occlusal conjunctiva (Figure 2.12)[184]. The evaluation of tear menisci is regarded as an indicator of the tear film volume[29, 31].

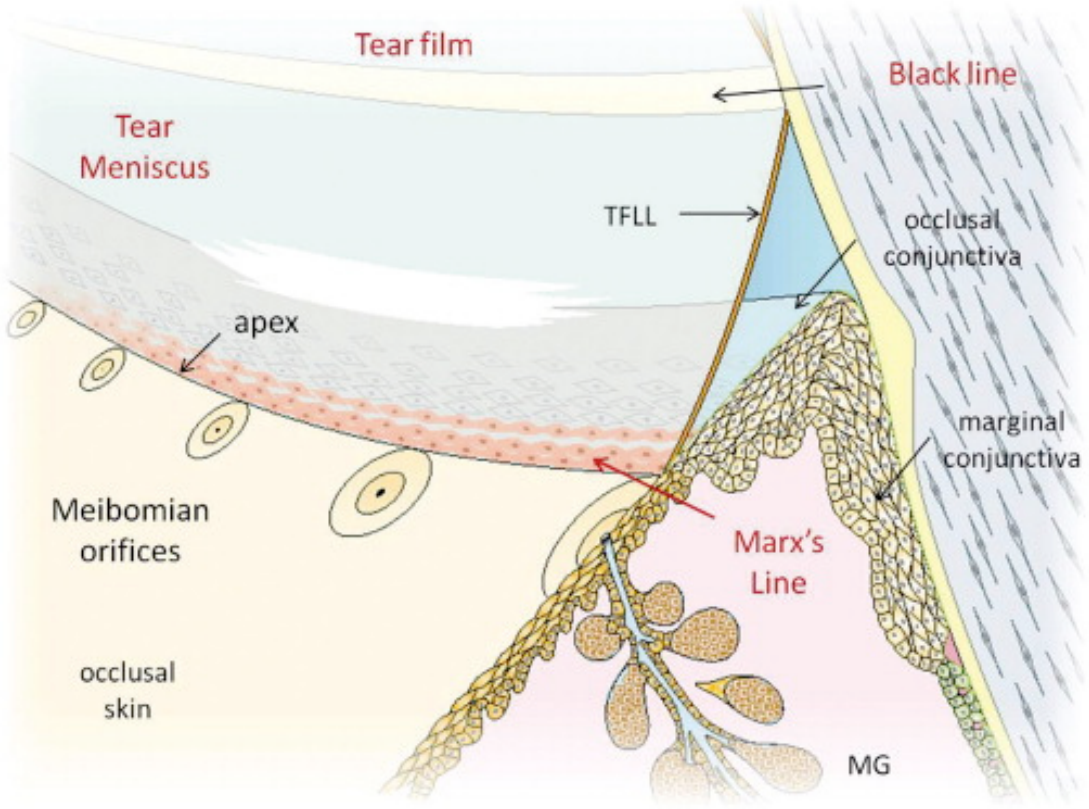


Figure 2.12: Schematic view of the lower tear meniscus and lid margin (from Bron et al. 2011)[184]. TFLL: Tear Film Lipid Layer; MG: Meibomian Gland.

The tear meniscus can be analysed by its height (TMH), curvature (TMR), area (TMA) or volume (TMV). TMV is calculated from the height (TMH), curvature (TMR) and length of the lid margin[177, 185].

All measurements should be performed while the patient is fixating on a target to maintain primary eye gaze. This is because the inferior TMH was found to be approximately 50% greater in 15° up-gaze than in primary eye gaze[186]. To avoid reflex tearing it is recommended to choose a low light intensity and to prevent direct shining of the light into the pupil[23, 128]. Temperature and humidity of the examination room were mentioned as being controlled in most of the recent studies[21, 24, 30, 31, 137, 143, 164, 169, 187-190]. Although there is no study about the influence of climate on TMH in the literature, Maruyama et al. found no significant difference in tear meniscus radius in an environmental chamber with the air temperature and relative humidity set at 5°C/10%, 15°C/20%, 25°C/40%, or 35°C/50%, indicating that the tear volume was not affected by air temperature and relative humidity over these environmental conditions[191]. On the other hand, they found a significant decrease in NIBUT with soft contact lenses as air temperature and relative humidity decreased. In addition, Purslow and Wolffsohn observed no significant correlation between TMH and ocular surface temperature[192].

2.2.1. Tear Meniscus Height

Measurement of tear meniscus height (TMH) is relatively easy and, when no fluorescein is used, it is a non-invasive method to determine the overall tear volume. Several techniques have been used to measure the TMH, including slit-lamp

evaluation with a graticule (Figure 2.13)[19, 20, 23, 37, 89, 193-198], variable slit-lamp beam height[16, 21, 168, 199], optical pachymetry[186, 200, 201], image capture methods[23, 24, 37, 100, 169, 187, 189, 200, 202, 203], video reflective dacryomeniscometry[143, 188] and optical coherence tomography[30-33, 137, 155, 156, 190, 200, 204, 205].

Because it might be difficult to define the top of the tear meniscus, some authors propose measuring the meniscus height not from the upper limit of the meniscus, but from the brightest reflex of the meniscus[22-24, 186]. Measuring in this way produces a lower height than the real TMH[22].

In other techniques, fluorescein is used to visualise the tear meniscus. However, while instillation of fluorescein enhances meniscus visibility, it causes the TMH to increase. It is therefore recommended to evaluate TMH 3 to 4 minutes after instillation, since TMH will have stabilised by that time[22, 195, 200], although some studies have demonstrated that useful levels of fluorescence last only for about 160 seconds and that fluorescein has washed out from the tear meniscus by 5 minutes post-instillation[22, 206].

The reported height of the inferior tear meniscus of healthy subjects varied between 0.12 and 0.37 mm (Table 2.2)[19, 20, 23, 37, 89, 193-195]. If the TMH is assessed in cross-section (Figure 2.14) with the slit-lamp, a mean of 0.15 mm has been found in normals[193], while with a front view technique (Figure 2.15), the mean was 0.21 mm[19, 20, 23, 89, 128, 194].

Author	Technique	Fluorescein	Lower TMH	Subjects	Age of Subjects
Papas and Vajdic[193]	Cross-section	No	0.15 ± 0.04	Normal (n=10)	
Tomlinson et al.[194]	Front view	No	0.35 ± 0.11	Normal (n=20)	30.6 years
Santodomingo-Rubido et al.[23]	Front view 40x	No	0.12 ± 0.05 *	Normal (n=55)	20.4 years
Miller et al.[20]	Front view 30x	No	0.25 ± 0.04	Normal (n=43)	25.0 years
			0.21 ± 0.09	Hydrogel	
			0.20 ± 0.04	Silicone-Hydrogel	
			0.24 ± 0.09	Gas-permeable	
Oguz et al.[37]	Cross-section	Yes	0.21 ± 0.14	Dry Eye (n=29)	60 ± 14.4 years
Lim and Lee[195]		No	0.19 ± 0.05	Normal	
			0.10 ± 0.04	Dry Eye	
Jordan and Baum[89]	Front view 25x	No	0.30 ± 0.06 †	Normal (n=15)	25 – 45 years
			0.23 ± 0.09 †	Normal (n=6)	57 – 71 years
Lamberts et al.[19]	Front view	No	0.23 ± 0.09	Normal (n=86)	
Patel and Wallace[128]	Front view 32x	No	0.19 ± 0.11	Normal female	51.9 ± 25.0 years
			0.19 ± 0.10	(n=268)	45.7 ± 27.7 years
				Normal male (n=176)	
Ibrahim et al.[196]	Front view	No	0.15 ± 0.05	Sjögren's syndrome	68.1 ± 13.4 years
				female (n=8)	
Garcia-Resua et al.[22]	Front view 40x	Yes	0.25 ± 0.08	Normal (n=34)	21.3 ± 2.1 years
			0.13 ± 0.05*		
Mean Normals			**0.22 ± 0.07**		

Table 2.2: Mean ± standard deviation of lower tear meniscus height values reported using graticule technique.

** Distance between the edge of the lower eyelid and the meniscus reflex, not the top of the meniscus.*

† Not given in the text of the paper. Values were measured as accurately as possible from the bar graph in the publication.

2.2.1.1. Graticule

Many clinicians measure TMH, using a graticule inserted into the slit-lamp eyepiece (Figure 2.13). The disadvantages of the graticule technique arise from the low magnification, the difficulty in defining the upper limit of the meniscus, and reflex tearing induced by the instillation of fluorescein[143]. Thus in a study by Santodomingo-Rubido et al., the repeatability of three consecutive measures at one visit with the graticule technique was 0.01 mm[23], but the repeatability of the TMH measurements taken at the two separated study visits was significantly greater (0.02 ± 0.05 mm, p = 0.01)[23]. Furthermore, the inter-observer reliability of the different examiners was found to be slight to moderate (Cohens kappa index 0.14 to 0.57), and the sensitivity and specificity were only 56% and 74%, respectively[207].

One reason for this might be that it is difficult for a practitioner to assess a TMH as small as 0.10 mm[208] when the graticule scale interval is only 0.10 mm[19]. Using a higher magnification of 32x will result in a better scale resolution of 0.03 mm[128].

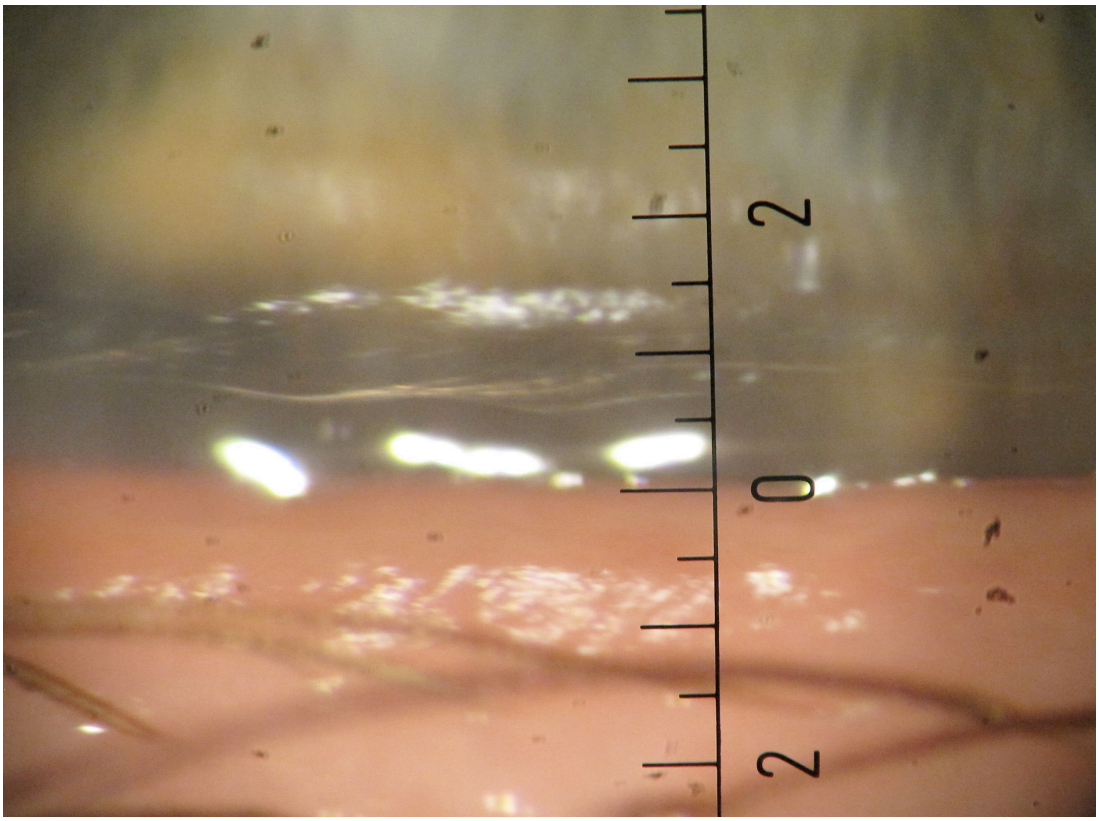


Figure 2.13: Graticule insert into slit-lamp eyepiece.

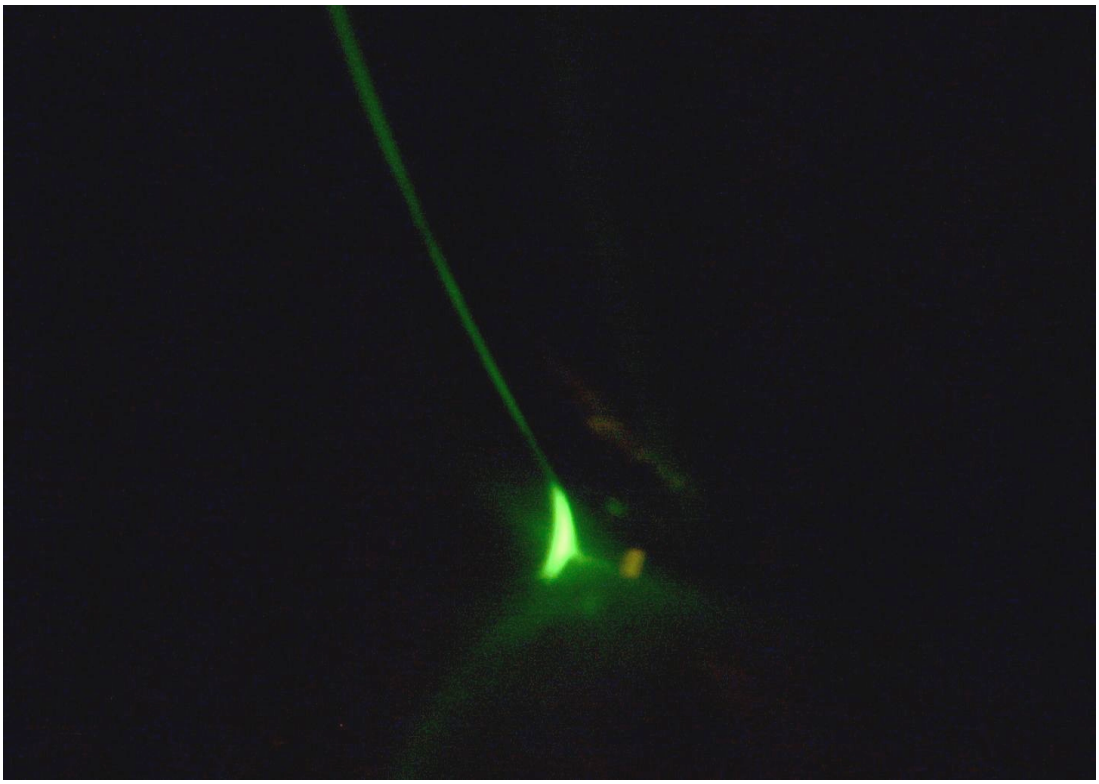


Figure 2.14: Fluorescein coloured tear meniscus in cross-section.

Figure 2.15: Tear meniscus in front view.

2.2.1.2. Variable Slit-Lamp Beam Height

Measurement with the slit-lamp can also be performed with the graduated slit opening mechanism[16, 21, 168, 199, 209]. The width of the beam in the horizontal or vertical position is set to equal the height of the tear meniscus (Figure 2.16), and the beam width can be read on the illumination system. Using the variable slit-lamp beam, Guillon et al. reported a TMH of 0.32 ± 0.11 mm on a group of asymptomatic non-lens wearers[168]. On a group of dry eye patients, TMH was found to be 0.29 ± 0.13 mm[21]. With this method a weak intra-class correlation coefficient (ICC) of 0.29, with a 95% confidence interval of (0.04, 0.51), was reported, which means this method shows great variation when it is performed by the same examiner on two occasions[21].

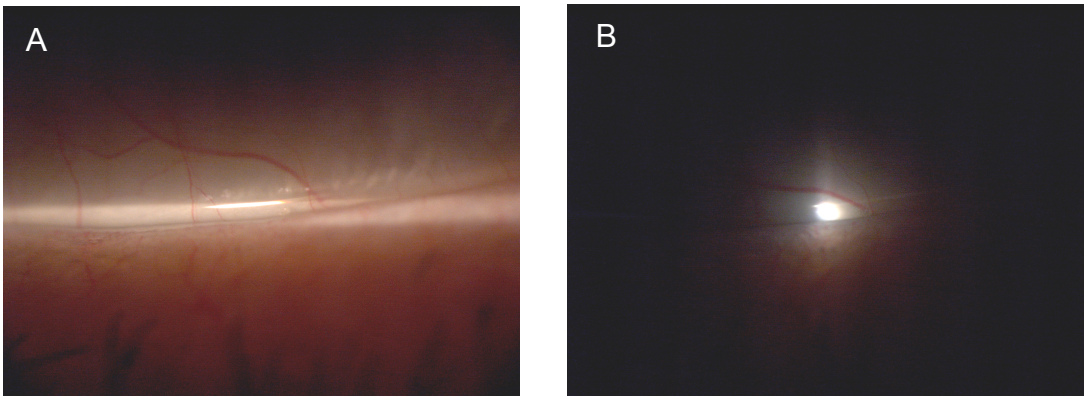


Figure 2.16: Measurement of TMH with variable beam height. Horizontal (A) or vertical (B) orientated.

2.2.1.3. Pachymetry

An optical pachymeter attached to a slit-lamp can also be used to obtain a measurement of TMH[186, 200, 201]. The slit-lamp optical pachymeter was originally designed to measure the thickness of the cornea using two plane parallel plates that bisect an optical section of the cornea. The plates separate the optical section into two halves, divided horizontally into upper and lower parts. One of the plates can then be rotated until the front corneal surface of one image section is aligned with the back of the second image. The amount of horizontal rotation necessary is proportional to the thickness of the cornea.

To apply the same principle to the image of the tear meniscus, the pachymeter has to be vertically orientated. The glass plate is rotated until the bottom of one image of the tear meniscus aligns the top of the second image of the meniscus. The separation of the images is then proportional to the height of the tear meniscus. With this technique the tear meniscus can be evaluated in cross-section (Figure 2.17) or in

front view (Figure 2.18). Depending on technique and use of fluorescein, mean TMH values of between 0.16 and 0.38 mm in normal healthy eyes have been reported (Table 2.3)[186, 200, 201]. However, the inter-test repeatability is poor in both techniques. Port and Asaria found a 95% repeatability coefficient of 0.03 mm for the optical pachymetry in front view[186], but Johnson and Murphy found 0.09 mm for front view and 0.19 mm for cross-section[200].

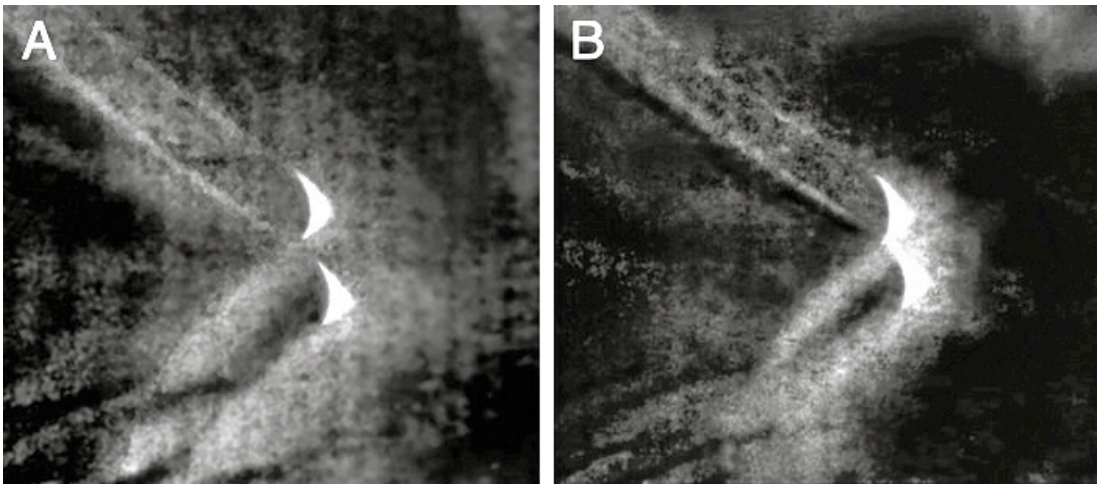


Figure 2.17: Doubling of the inferior tear meniscus when viewed in cross-section with an optical pachymeter (A). The end point with this measurement technique (B) (from Johnson and Murphy 2005)[200].

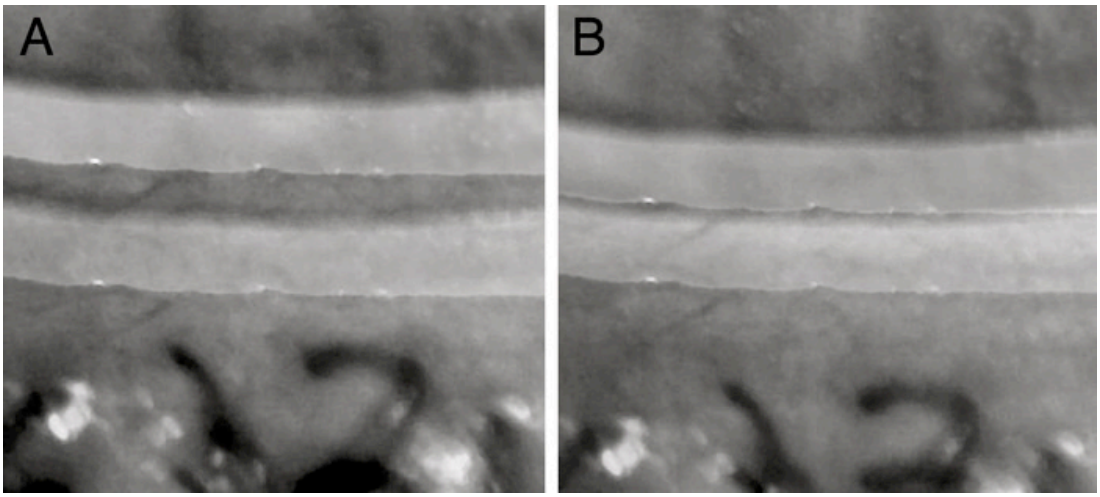


Figure 2.18: The view obtained when using an optical pachymeter attached to a slit lamp to measure the inferior tear meniscus height from the front (A). Rotation of one of the glass plates of the pachymeter aligns the top and bottom of the two menisci images (B). The angular separation of the two glass plates at this point is proportional to the height of the meniscus (from Johnson and Murphy 2005)[200].

Author	Technique	Fluorescein	Lower TMH	Subjects	Age of subjects
Johnson and Murphy[200]	Cross-section	No	0.33	Normal (n=25)	27.0 years
		Yes	0.31		
	Front view	Yes	0.38		
	Cross-section				
Patel and Port[201]	Front view	No	0.16*	Normal (n=5)	25 to 46 years
			0.25*	Visual Display Unit Users (n=5)	21 to 43 years
Port and Asaria[186]	Front view	No	0.18*	Normal (n=66)	16 to 25 years
Mean Normals			**0.27**		

Table 2.3: Mean of lower tear meniscus height values reported using pachymetry technique.

** Distance between the edge of the lower eyelid and the meniscus reflex, not the top of the meniscus.*

2.2.1.4. TMH Image Capture

In normal eyes, mean TMH values of between 0.13 and 0.46 mm have been reported for the inferior meniscus[23, 24, 37, 100, 169, 187, 189, 200, 202, 203, 210] and 0.36 mm for the superior meniscus[100] using an image capture technique (Table 2.4). The image of the meniscus can either be taken by a video camera with a capture mode or a digital camera. Images are analysed using the printout of the photographs or directly on the video-screen. A ruler or image analyser software is applied to measure the millimetres or the pixels of tear meniscus on the image (Figure 2.19). Santodomingo-Rubido et al.[23] found the image capture technique to be more repeatable than the graticule technique and attributed this to the higher measurement resolution of the image capture (0.0018 mm) compared to the graticule technique (0.03 mm). As before, the meniscus is viewed in cross-section or front-view, and with or without the use of fluorescein. The use of a tear interference device (Tearscope Plus™) in combination with image analysis software is described by Uchida et al.[210]. They found that the interference phenomena could visualise the TMH even when it was very low. The tear analysis software (TF-scan) on the Oculus Keratograph (Oculus Optikgeräte GmbH, Wetzlar, Germany) also allows the measurement of TMH with an integrated calliper in the image capture mode of the instrument[211, 212].

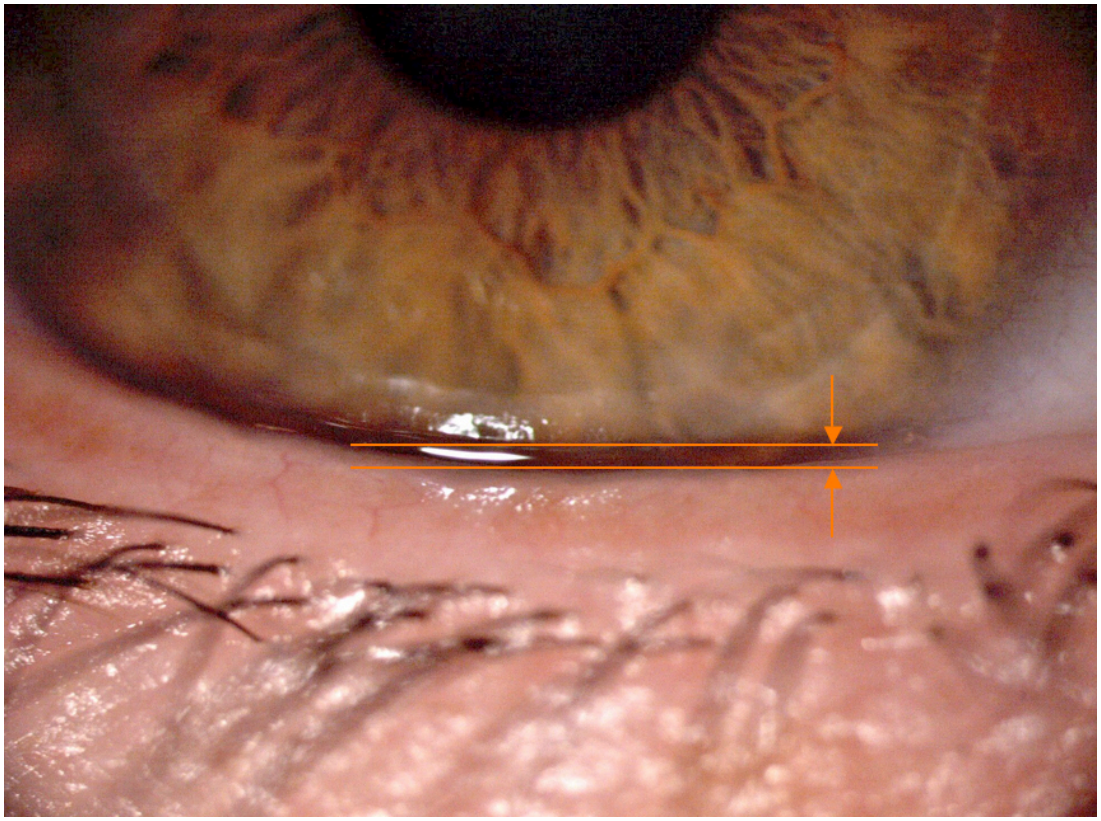


Figure 2.19: Image captured for analysing tear meniscus height in front view, using the calliper tool in ImageJ software.

Author	Technique	Fluo-rescein	Lower TMH	Upper TMH	Subjects	Age of subjects
Harrison et al.[100]	Videotaped with a millimetre ruler Front view	Yes	0.40 ± 0.09 0.30 ± 0.07	0.36 ± 0.07 0.27 ± 0.08	Normal (n= 15) Dry Eye (n=15)	33 ± 12 33 ± 13
Santodomigo-Rubido et al.[23]	Win TV Pixels in mm Front view	No	0.13 ± 0.04 *		Normal (n=55)	20.4
Johnson and Murphy[200]	ImageNET 2000 Front view	Yes	0.34 ± 0.05		Normal (n=25)	27.0
Glasson et al.[169]	Specular reflection Cross section	No	0.43 ± 0.11 0.31 ± 0.09		CL tolerant (n=20) CL intolerant (n=17)	21-38 25-39
Doughty et al.[24]	Video-recorder, Precision ruler. Front view	No	0.19 ± 0.09 *		Normal (n=97)	> 60
Oguz et al.[37]	Video-recorder, Videoprinter Cross section	Yes	0.24 ± 0.09		Dr Eye (n=29)	60 ± 14.4
Zaman et al.[202]	Video image		0.18 ± 0.11		Normal (n=45)	70.8 years
Golding et al.[203]	NIH Software Cross section	Yes	0.42 ± 0.16		Normal and Dry Eye (n=30)	65.6 ± 10.2
Mainstone et al.[189]	Kodak 400 ASA NIH Software Cross section	Yes	0.46 ± 0.17 0.24 ± 0.09		Normal (n=15) Dry Eye (n=15)	64.4 ± 11.1 65.1 ± 13.4
Uchida et al.[210]	ImageJ Software Tearscope Plus Front view	No	0.22 ± 0.07 0.13 ± 0.42		Normal (n=15) Dry Eye (n=27)	52 ± 16 62 ± 10
Finis et al.[213]	TF Scan Oculus Keratograph	No	0.40 ± 0.30		MGD (n=17)	45 ± 23
Mean Normals			**0.28 ± 0.09**	**0.36 ± 0.07**		

Table 2.4: Mean ± standard deviation of lower and upper tear meniscus height values reported using videocapture techniques. * *Distance between the edge of the lower eyelid and the meniscus reflex, not the top of the meniscus.*

2.2.1.5. Videoreflective Dacryomeniscometry

Videoreflective dacryomeniscometry is carried out by imaging the specular reflection of a grid consisting of parallel black and white lines from the tear meniscus (Figure 2.20)[143, 188]. The images of the tear meniscus are then measured with digital screen callipers. An advantage of this method is that the specular reflection allows better definition of the top of the meniscus[143]. Francis et al. and Stahl et al. applied this method to patients with nasolacrimal duct obstructions and found significantly greater TMH (medians of 0.62 and 0.73 mm), which was reduced by lacrimal drainage surgery[143, 188]. The method was originally developed by Ho et al.[214] for imaging and describing the profile of the tear meniscus.

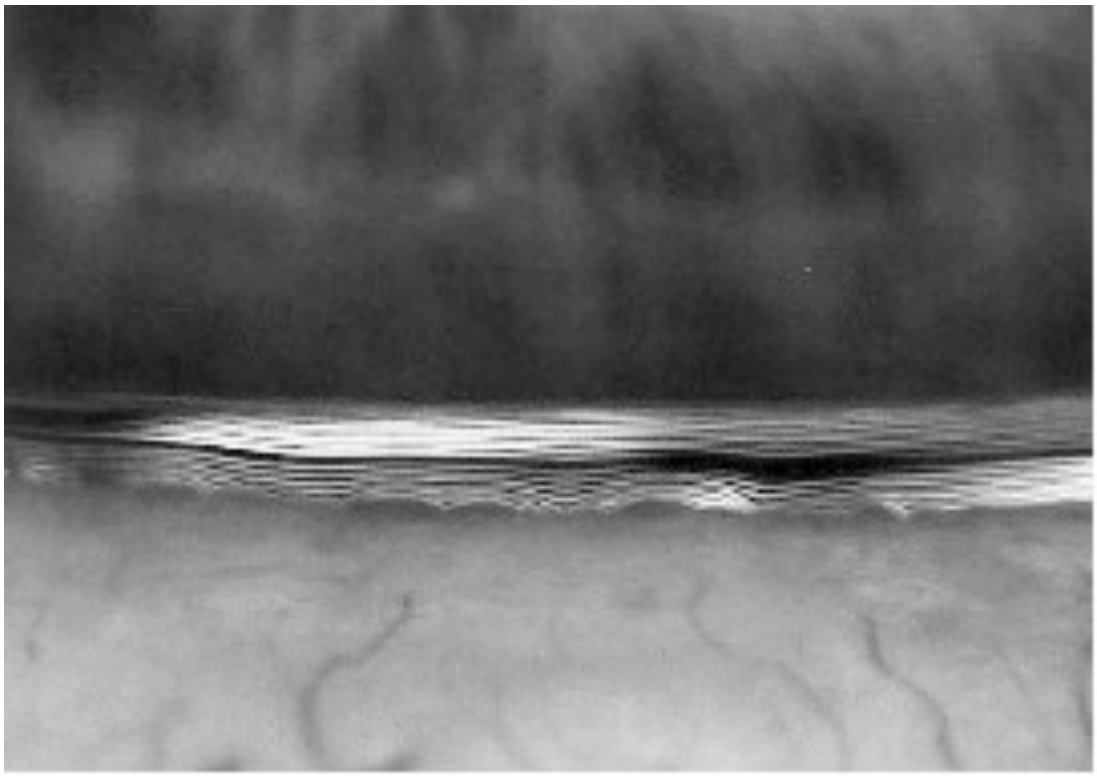


Figure 2.20: Typical appearance of marginal tear film using videoreflective dacryo-meniscometry in a patient with left primary acquired nasolacrimal duct obstruction post dacryocystorhinostomy (from Francis et al. 2005)[143].

2.2.1.6. Optical Coherence Tomography

Optical coherence tomography (OCT) is an optical signal acquisition and processing method allowing extremely high quality, micrometre resolution, three-dimensional images from within optical scattering media to be obtained. At first it was primarily used to obtain non-invasive images of the posterior segment of the eye *in vivo*. Different layers within the retina can be differentiated and retinal thickness can be measured. With some modifications and additional customised software, it is possible to apply these instruments to the anterior segment[215]. New anterior segment optical coherence tomographers have been introduced to the market with an axial resolution between 11 and 18μm[216-218] (Figure 2.21). Compared to the OCTs that were designed for the posterior segment, no additional external application is necessary to measure TMH[205]. They potentially offer a new, fast and easy method for assessing TMH because internal software enables direct measurements of the captured images, but they are not interchangeable with the posterior segment OCTs[190].

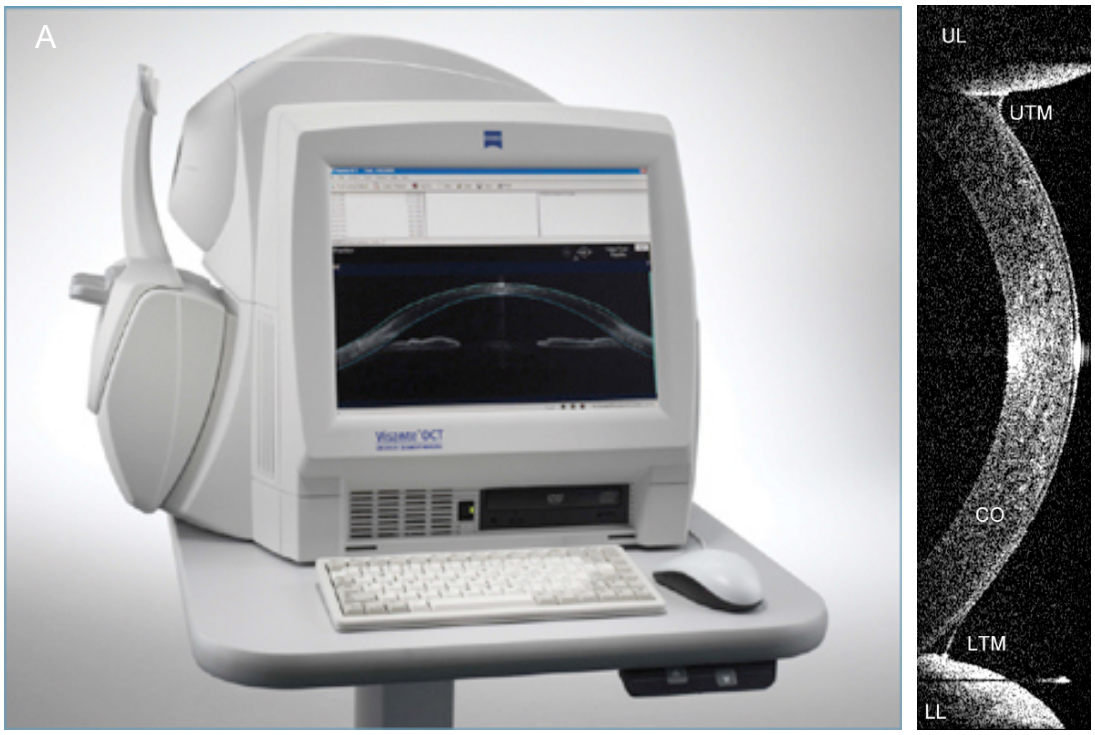


Figure 2.21: (A) Zeiss Visante® optical coherence tomographer for anterior segment of the eye (Carl Zeiss Meditec AG, Jena, Germany). (B) OCT image of the lower (LTM) and upper tear meniscus (UTM) (from Shen et al., 2009)[28].

OCT devices are currently classified into 3 types of systems: Time Domain (TD) OCT systems, Spectral Domain (SD) OCT systems, and Swept Source (SS) OCT systems (Figure 2.22).

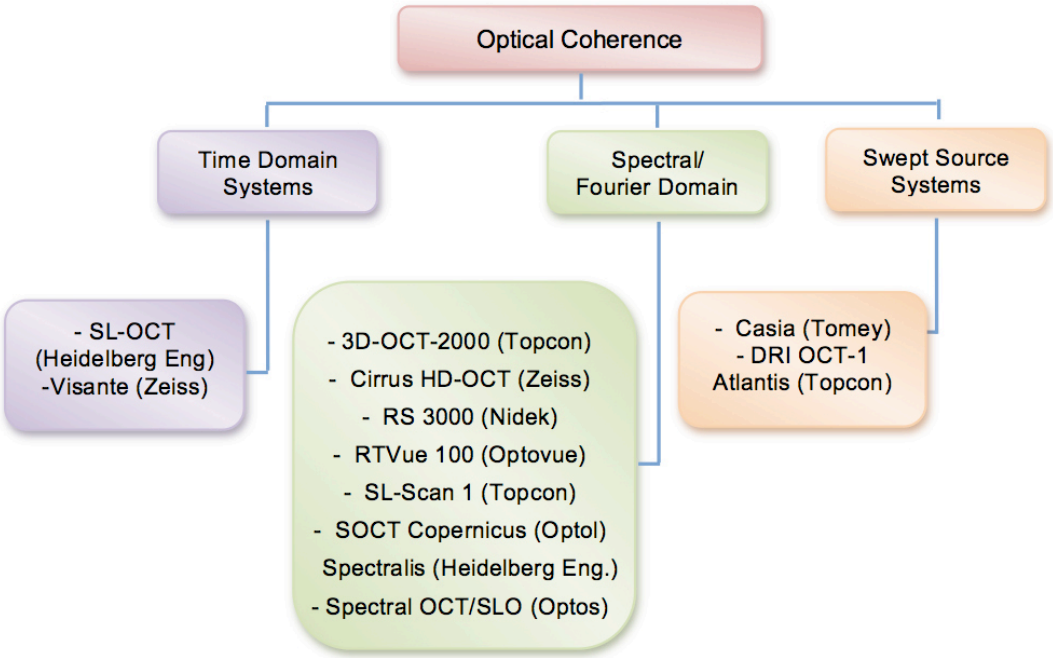


Figure 2.22: Different types of optical coherence tomography systems.

The Time-Domain OCT systems belong to the first generation of OCT devices. With these, a variable group delay reference arm was used to coherently gate backscattered light from various depths in a sample[219]. In contrast to the TD-OCT systems, the newer Spectral Domain OCT systems have a fixed reference path, and the signal coming back from the tissue is analysed by a spectrometer. Since no mechanical adjustment of the reference path is required with this system, and the incoming signals can be processed at the same time, the SD-OCT systems offer significantly better axial resolutions. The latest enhancements of OCT technology are

the Swept Source OCT systems[220]. In this case, a wavelength-adjustable laser is used, which allows extremely fast and low-noise signal processing. Due to the shorter scan duration, high-resolution, real-time images, for example of an accommodating lens or the movement of a contact lens on the eye, can be captured[221, 222].

The dimensions of the images produced by an OCT do not, however, correspond with reality. There are underlying distortions in the images, which means that dimensions cannot be measured accurately. One of these distortions is the "Fan-distortion", which is created by the design of the scanner, and the arrangement and design of the mirror and the collimator lens[223, 224]. This has the effect that a flat surface appears to be bent. Further distortions, called "optical distortions", are caused by variations in the refractive indices of the tissue to be measured[223, 224]. The higher the refractive index of the tissue, the longer it takes for the light to be transmitted by the tissue. The result of these distortions is that a measuring scale, calibrated to measure corneal thickness, cannot be used to measure other tissue structures. In order to perform reliable measurements with the OCT, despite the resulting distortions, software algorithms are required to eliminate the errors (Figure 2.23) [225, 226].

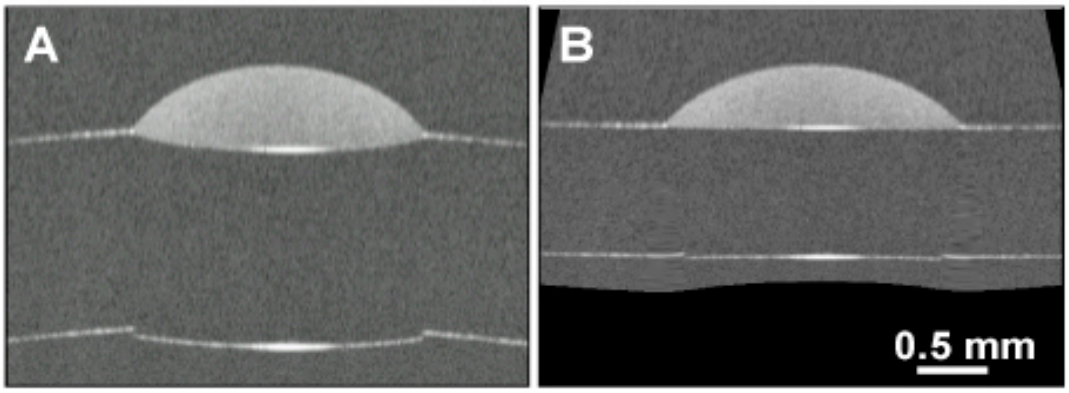


Figure 2.23: (A) Uncorrected OCT image of a lipid drop on a glass microscope cover slip, (B) corrected image (by Westphal et al. 2002)[225].

Measured TMH with the different OCTs varies between 0.14 and 0.34 in normal healthy subjects (Table 2.5). Analysing TMH on the images of one OCT by two different observers shows differences that can be minimised by training sessions[204]. A moderate intra-class correlation coefficient of 0.605 was found for TMH with a recently developed Fourier-domain optical coherence tomographer[227].

Author	Technique	Lower TMH	Upper TMH	Subjects	Age of subjects
Savini et al.[190]	Zeiss Visante	0.28 ± 0.12		Dry eye and normal (n=26)	32.4 ± 6.1 years
	Zeiss Stratus	0.23 ± 0.07			
Shen et al.[137]	Real-time OCT	0.28 ± 0.08	0.20 ± 0.04	Normal (n=20) 1:00 PM	21.3 ± 1.5 years
Wang et al.[33]	Real-time OCT			CL wearers (n=20)	31.7 ± 8.9 years
		0.26 ± 0.05	0.27 ± 0.04	Balafilcon A	
		0.28 ± 0.07	0.28 ± 0.05	Galyfilcon A	
Wang et al.[30]	Zeiss OCT-?	0.34 ± 0.15	0.27 ± 0.05	Normal (n=36)	45.1 ± 15.4 years
Srinivasan et al.[156]	Zeiss OCT-1	0.14 ± 0.02		Normal (n=20)	27.0 ± 6.0 years
		0.13 ± 0.01		Dry eye (n=20)	
Bitton et al.[204]	Zeiss OCT-2	0.25 ± 0.08		Normal (n=10)	32.5 ± 6.4 years
Palakuru et al.[31]	Zeiss OTC-?			Normal (n=21)	32.1 ± 8.7 years
		0.31 ± 0.09	0.26 ± 0.05	Before blink	
		0.30 ± 0.08	0.25 ± 0.04	After blink	
Wang et al.[32]	Zeiss OTC-?	0.26 ± 0.08	0.27 ± 0.07	Normal (n=20)	40.5 ± 14.1 years
Savini et al.[205]	Zeiss OCT-3	0.13 ± 0.07		Dry eye (n=27)	51.2 ± 16.4 years
		0.25 ± 0.08		Normal (n=20)	36.8 ± 10.9 years
Johnson and Murphy[200]	Zeiss OCT-1	0.27		Normal (n=25)	27.0 years
Qiu et al.[133]	Optovue	0.40 ± 0.11		Normal (n=6)	0-19 years
	RTVue	0.32 ± 0.08		Normal (n=60)	20-39 years
		0.30 ± 0.11		Normal (n=60)	40-59 years
		0.28 ± 0.10		Normal (n=20)	>59 years
Czajkowski et al.[228]	Optovue	0.34 ± 0.11		Normal (n=56)	34.4 ± 11.21 years
	RTVue	0.20 ± 0.08		Dry eye (n=13)	
Tung et al.[229]	Optovue	0.35 ± 0.18		Normal (n=33)	51.0 years
	RTVue	0.31± 0.16		MGD (n=23)	57.0 years
		0.21 ± 0.09		ATD (n=41)	65.0 years
Napoli et al.[230]	Zeiss Cirrus	0.27 ± 0.07		Normal (n=15)	49.2 ± 2.48 years
Fukuda et al.[231]	Tomey	0.26 ± 0.06	0.23 ± 0.08	Normal (n=26)	36.5 ± 6.8 years
	SS-1000				
Mean Normals		**0.27 ± 0.09**	**0.24 ± 0.07**		

Table 2.5: Mean ± standard deviation of lower and upper tear meniscus height values reported using optical coherence tomography.

2.2.2. Tear Meniscus Radius of Curvature

Measurement of the tear meniscus radius (TMR) can be performed with meniscometry[26, 27, 29, 34, 37], with a slit-lamp image capture technique[163, 189, 203, 232], or with optical coherence tomography[28, 30, 31, 33, 137, 164, 170, 173, 177]. A significant positive correlation (r=0.596, p=0.0005) has been reported between TMH and TMR, thus a smaller TMR can be expected in eyes with lower tear volumes[37, 203].

2.2.2.1. Reflective Meniscometry

Reflective meniscometry is a non-invasive technique to measure the tear meniscus radius by projecting a target on to the meniscus. The target consists of horizontal black and white stripes positioned at a given angle to an observation system to produce specular reflection of the tear meniscus strip (Figure 2.24). The tear meniscus acts as a concave mirror and creates an image of the stripes on the meniscus (Figure 2.25). The image can be manually analysed on a printout, or the digital image can be analysed by software. Digital image analysis of the reflective image shows a high correlation with manual image analysis and shortens the measurement period by 33%[233]. The distance between the black and white lines can be measured for a given magnification and the radius of the meniscus can be calculated with the concave mirror formula (Table 2.6).

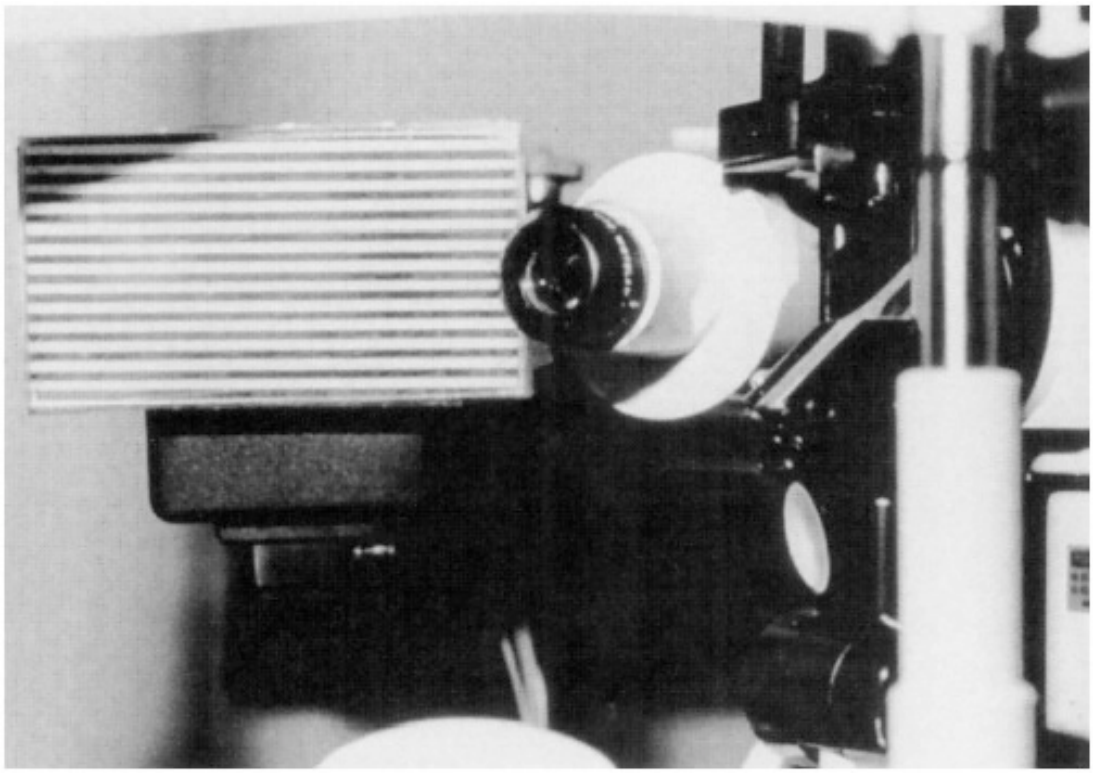


Figure 2.24: Reflective meniscometer presented by Yokoi et al. in 1999.[27]

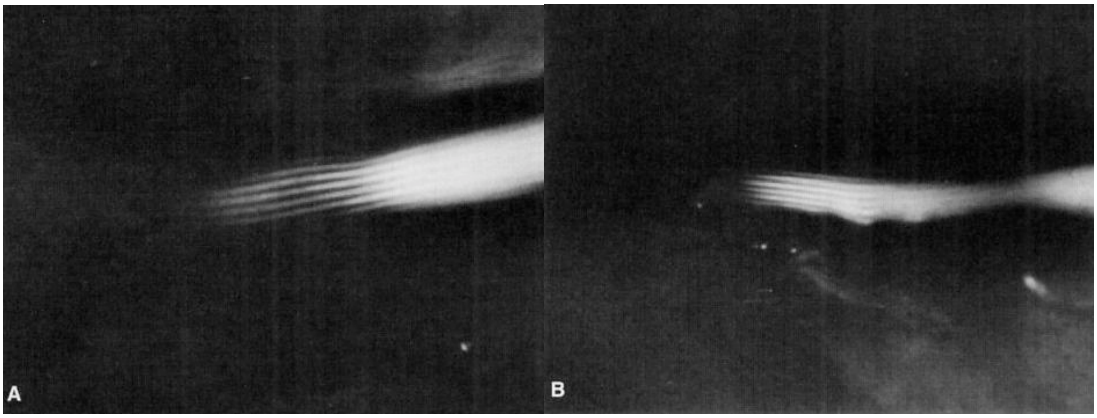


Figure 2.25: Representative images of the meniscus obtained with reflective meniscometry. (A) Normal eye; and (B) dry eye (from Yokoi et al. 2000)[34].

The first photographic meniscometer was introduced by Bron et al.[234, 235] in 1997. It consists of a target of 14 black and 13 white lines, each 2 mm wide, attached to a macro-camera (Figure 2.24)[27]. A video system with a CCD camera and target consisting of a central white bar of 3.5 mm wide on a black surround was also described. A modification of the video system, called a "video-meniscometer", with a target of a series of black metal bars, 4 mm wide and 4 mm apart, set right in front of the objective lens and illuminated from behind was developed by Oguz et al.[37] and Yokoi et al.[34] (Figure 2.26).

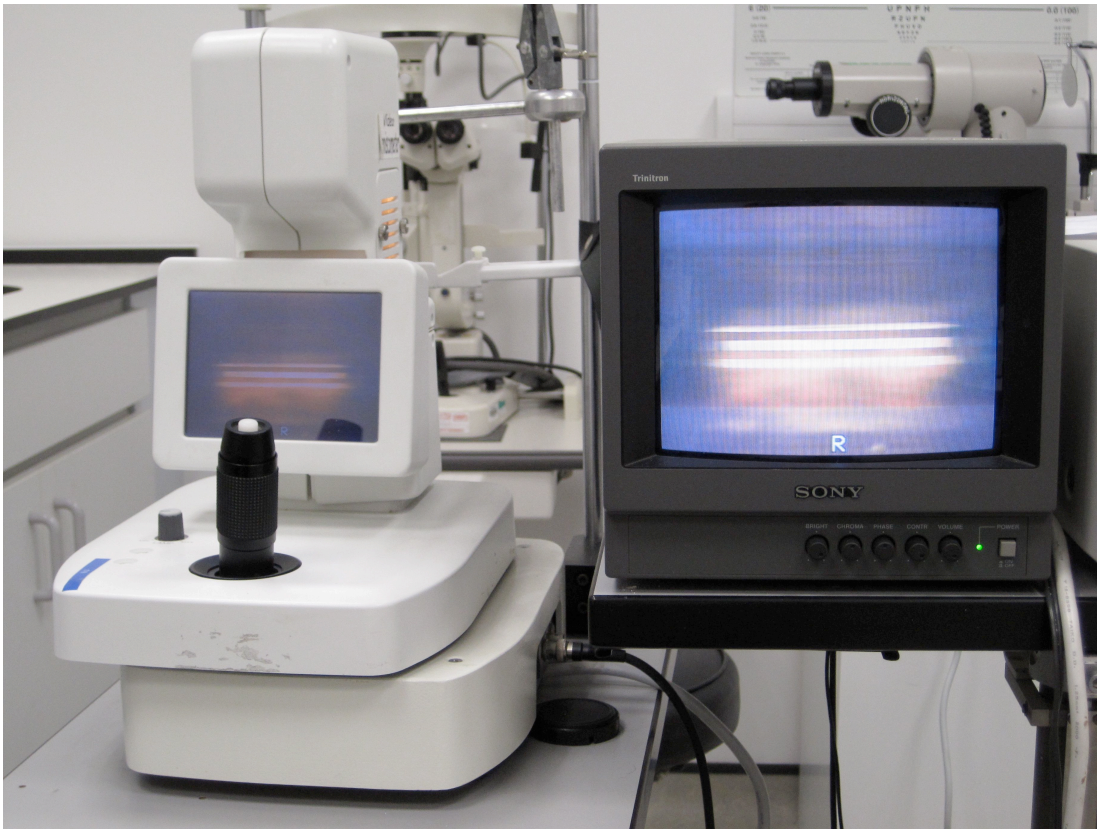


Figure 2.26: The Bron Videomeniscometer.

A method similar to reflective meniscometry was described by Ho et al. and named dacryomeniscometry[214]. They modified a Zeiss SL-30 biomicroscope by adding an illuminated horizontal grid of black and white lines, 1 cm wide in front of the observation system, 150 mm from the subject's eye, and used two different CCD-cameras to record the image of the grid on the tear meniscus. Furthermore, they assumed the meniscus to be an aspheric curve lying in a vertical plane and developed an algorithm to compute the meniscus profile from the images. As reference, samples of copper wire of known cross-sectional radius, which were pressed into a heated block of clear acrylic, were used. The application of this method to a group of 13 normal subjects revealed considerable day-to-day variations in tear meniscus radius, and nine of the thirteen subjects showed a greater mean radius in the morning, although it failed to reach statistical significance ($p=0.06$)[214]. Beside this study, dacryomeniscometry was used in two additional studies to evaluate the height of the tear meniscus[143, 188].

Magnification of the system	$M = \frac{y'_a}{y'_b}$	y'_a = image size with the instrument (measured on printout or computer screen) y'_b = image size without the instrument (real image size)
Radius of meniscus	$r = \frac{2 \cdot a \cdot y'}{y - y'}$	r = radius of meniscus curvature a = target distance y = target size y' = image size

Table 2.6: Formulae for calculating the magnification of the system and the radius of meniscus curvature.

The measurement of the tear meniscus radius by meniscometry has several advantages over other methods:

(1) There is no fluorescein needed to evaluate the tear film so the method is non-invasive.

(2) Meniscometry provides a clear and stable image of the tear meniscus, so there are no difficulties in confirming the upper extremity of the meniscus, which is one of the main problems in tear meniscus height measurement.

(3) By using video-meniscometer, real time dynamic changes of the tear meniscus profile, e.g. after a blink or eye drop instillation, can be made visible.

(4) Meniscometry shows high precision for measurement of even small radii of different glass capillaries (r^2=0.996, p<0.0001)[27].

(5) The tear meniscus radius is correlated with tear volume, so meniscometry is an effective tool in the diagnosis of aqueous-tear deficiency[28, 29].

(6) Meniscometry measurements are repeatable; there was no significant difference in curvature between two consecutive photographs of the meniscus taken at an interval of 20 seconds (p=0.847 (paired *t* test); first photograph: 0.365 ± 0.153 mm, second photograph: 0.367 ± 0.132 mm[27].

Mean TMR values of 0.37 mm for normal and of 0.22 to 0.25 mm for dry eye patients were reported (Table 2.7).

Author	Technique	Lower TMR	Subjects	Age of subjects
Yokoi et al.[29]	4 black and 5 white lines (each 4 mm wide) in the optical pathway, 70x (Video-Meniscometer)	0.24 ± 0.08	Dry Eye (n=11)	66.2 ± 7.7
Yokoi et al.[34]	14 horizontal black and 13 white stripes (each 2 mm wide) 40° target/camera	0.37 ± 0.15 0.25 ± 0.09	Normal (n=45) Dry Eye (n=32)	45.6 ± 21.0 55.3 ± 17.4
Oguz et al.[37]	4 black and 5 white lines (each 4 mm wide) in the optical pathway, 70x (Video-Meniscometer)	0.22 ± 0.09	Dry Eye (n=29)	60 ± 14.4
Yokoi et al.[27]	14 horizontal black and 13 white stripes (each 2 mm wide) 40° target/camera	0.37 ± 0.15	Normal (n=45)	45.6 ± 21
Maruyama et al.[191]	4 black and 5 white lines (each 4 mm wide) in the optical pathway, 70x (Video-Meniscometer)	0.26 ± 0.09*	Normal (n=11)	23.5 ± 5.2
Watanabe et al. [236]	4 black and 5 white lines (each 4 mm wide) in the optical pathway, 70x (Video-Meniscometer)	0.31± 0.16 0.23± 0.08	Before (n=36) After blephar-optosis repair	66.3 ± 12.1
Yokoi et al.[237]	4 black and 5 white lines (each 4 mm wide) in the optical pathway, 70x (Video-Meniscometer)	0.30± 0.02	Normal (n=20)	38.8 ± 6.9
Mean Normals		**0.35 ± 0.11**		

Table 2.7: Mean ± standard deviation of lower tear meniscus radius values reported using reflective meniscometry.

* *Average of measurements under four different environment conditions.*

2.2.2.2. TMR Image Capture

Like the evaluation of tear meniscus height, the radius of the meniscus can be observed in cross section with the slit-lamp, with sodium fluorescein instilled in the tear film to improve visibility of the meniscus[163, 189, 203, 232]. Just as with TMH, the image of the meniscus is captured and the radius is analysed with different image software packages. TMR is assessed by determining the radius of a circle that best

fits the curved anterior meniscal surface (Figure 2.27)[189, 203, 232], or by marking the top and the bottom of the meniscus and then adding three additional points with equal vertical spacing (five points in total)[163]. By finding a circle that passes through three of these points, the radius of curvature for the upper and lower parts of the meniscus can be determined. Using this method, it has been shown that, following a blink, the meniscus rapidly becomes eccentric, with the radius of the upper half exceeding that of the lower by 0.19 mm[163].

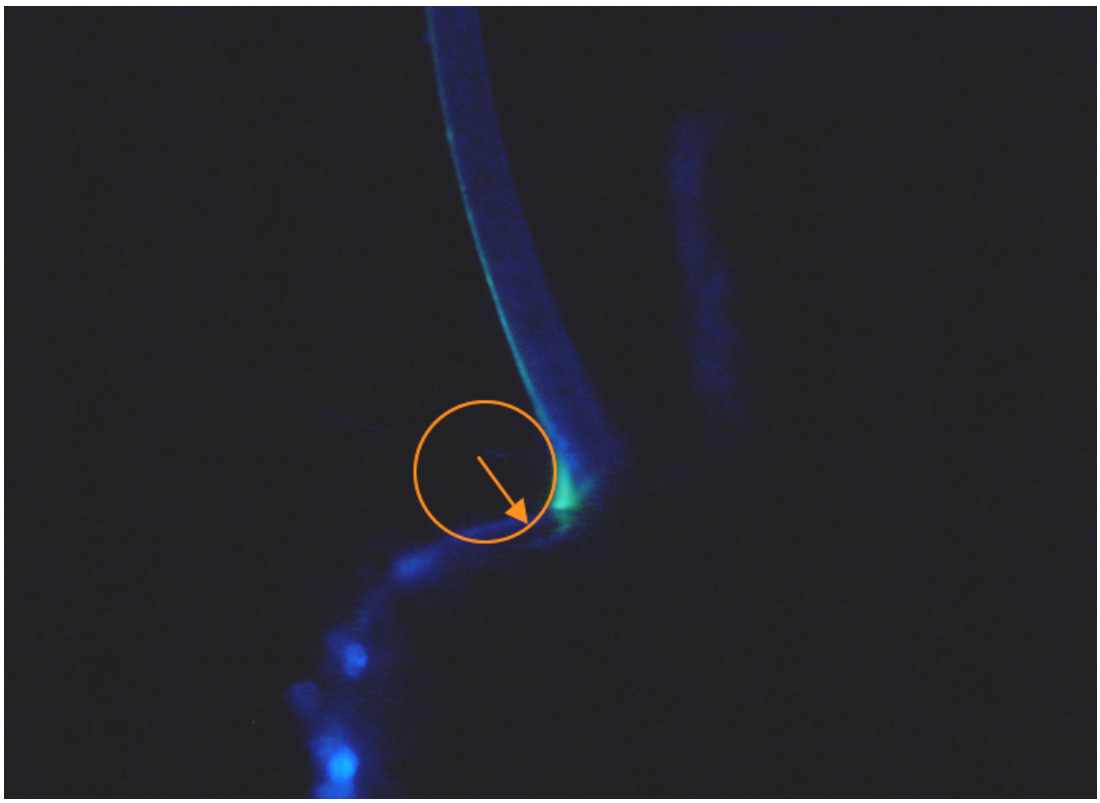


Figure 2.27: Image capture for analysing tear meniscus radius in cross section.

TMR in normals, with the image capture technique, varies between 0.33 and 0.55 mm[163, 189, 203] (Table 2.8). The image capture technique for measurement of the TMR shows good reliability[203] and good diagnostic accuracy, with a dry eye referent value of $\leq$ 0.35 mm[189]. However, the values of TMR with the image capture technique are

greater than that reported with meniscometry, and probably reflect the invasive nature of the present method, since fluorescein instillation is required.

Author	Technique	Fluorescein	Lower TMR	Subjects	Age of subjects
Johnson and Murphy[163]	Cross-section PowerPoint, Circle passes three given points.	Yes	0.33 direct after blink to 0.48 after 10 s later (Average of lower and upper TMR)	Normal (n=15)	29
Creech et al.[232]	Cross-section NIH Image software.	Yes		Normal (n=24) Hydrogel (n=15) RGP (n=6)	29 ± 8
Golding et al.[203]	Cross-section, endothelial attachment, NIH Image software	Yes	0.48 ± 0.21 Visit 1 0.42 ± 0.17 Visit 2	Normal and Dry Eye (n=30)	64.4 ± 11.1 65.1 ± 13.4
Mainstone et al.[189]	Cross-section, endothelial attachment, NIH Image software	Yes	0.55 ± 0.26 0.24 ± 0.09	Normal (n=15) Dry Eye (n=15)	64.4 ± 11.1 65.1 ± 13.4
Mean Normals			**0.50**		

Table 2.8: Mean ± standard deviation of lower tear meniscus radius values reported using image capture technique.

2.2.2.3. Optical Coherence Tomography

Wang et al.[238] used a full-field real-time OCT, developed by Radhakrishnan et al.[239], to image the superior and inferior tear menisci at the same time, with an optical resolution of <10 μm. To extract the radius of curvature from the OCT images, the

3-point method was used to fit a circle. The average TMR in normals reported with this method was 0.34 ± 0.23 mm for the inferior, and 0.24 ± 0.08 mm for the superior tear meniscus (Table 2.9).

Author	Technique	Lower TMR	Upper TMR	Subjects	Age of subjects
Wang et al.[30]	Real-time OCT	0.46 ± 0.40	0.25 ± 0.08	Normal (n=36)	45.1 ± 15.4 years
Palakuru et al.[31]	Real-time OCT			Normal (n=21)	32.1 ± 8.7 years
		0.39 ± 0.31	0.26 ± 0.10	Before blink	
		0.36 ± 0.31	0.25 ± 0.10	After blink	
Wang et al.[32]	Real-time OCT	0.26 ± 0.15	0.24 ± 0.11	Normal (n=20)	40.5 ± 14.1 years
Wang et al.[33]	Real-time OCT	0.29 ± 0.16	0.25 ± 0.07	Normal (n=20)	31.7 ± 8.9 years
				After CL Insertion:	
			0.29 ± 0.11	Balafilcon A	
			0.30 ± 0.09	Galyfilcon A	
Shen et al.[28]	Real-time OCT	0.25 ± 0.05	0.21 ± 0.04	Normal (n=47)	38.5 ± 12.7 years
		0.15 ± 0.03	0.16 ± 0.03	Dry Eye (ATD) (n=48)	38.6 ± 13.2 years
Li et al.[240]	Real-time OCT	0.24 ± 0.05	0.20 ± 0.04	Normal (n=48)	33.3 years
		0.15 ± 0.04	0.15 ± 0.03	Dry Eye (n=50)	35.2 years
Napoli et al.[230]	Zeiss Cirrus	0.50 ± 0.02		Normal (n=15)	49.2 ± 2.48 years
Mean Normals		**0.34 ± 0.18**	**0.24 ± 0.08**		

Table 2.9: Mean ± standard deviation of lower and upper tear meniscus radius values reported using optical coherence tomography.

The values of the inferior TMR in normals with the OCT (0.34 ± 0.18 mm) is similar to those measured with meniscometry (0.35 ± 0.11 mm) (Table 2.7 and 2.9), which might reflect the non-invasive nature of both methods where no fluorescein instillation is required. Like meniscometry, OCT requires expensive instrumentation and at present the application seems to be more frequent in laboratories than in clinical practice.

2.2.3. Tear Meniscus Cross-Sectional Area

The tear meniscus is bordered by the lid margin, the surface of the cornea or bulbar conjunctiva and, at the front, by air. In simplified view, the cross-sectional area of the meniscus forms a triangle (Figure 2.28). The prism area of the triangular shaped meniscus can then be calculated. Applying this method, Glasson et al.[169] found a significant difference in tear meniscus area between tolerant and non-tolerant soft contact lens wearers (0.07 mm^2 versus 0.04 mm^2).

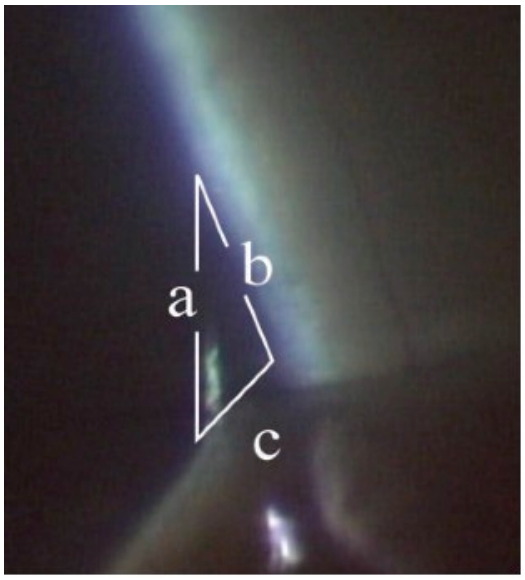


Figure 2.28: Cross-sectional area of meniscus = (√[s(s – a)(s – b)(s – c)]) /(magnification factor)^2, where s = (a + b + c)/2 (from Glasson et al. 2003)[169].

Another method to calculate the cross-sectional area of the meniscus is to capture an image of the fluorescein coloured meniscus and analyse it using image software with an outlining and threshold tool. Employing this method, Mainstone et al.[189] reported areas of 0.018 mm^2 for normal and of 0.008 mm^2 for dry eye subjects. In recent

studies, OCT was used to obtain a cross-sectional image of the tear meniscus without the need for fluorescein. Three points were marked on the OCT image of the meniscus: the touch points between tear film and cornea, tear film and eyelid, and one middle point of the front edge of the tear meniscus (Figure 2.29).

As described earlier, the 3-point method is used to fit a circle to yield the meniscus radius. Thus, the meniscus height is taken from the same image, and the cross-sectional meniscus area can be calculated. Wang et al.[32] calculated the tear meniscus area of the inferior and superior menisci from OCT images of healthy subjects and found an area of 23999.5 μm^2 for the inferior and 22731.5 μm^2 for the superior menisci. Smaller values of 15927 μm^2 for the inferior and 12609 μm^2 for the superior menisci were reported by Shen et al.[28]. They found the tear meniscus cross-sectional areas to be significantly smaller in dry eye patients than healthy subjects.

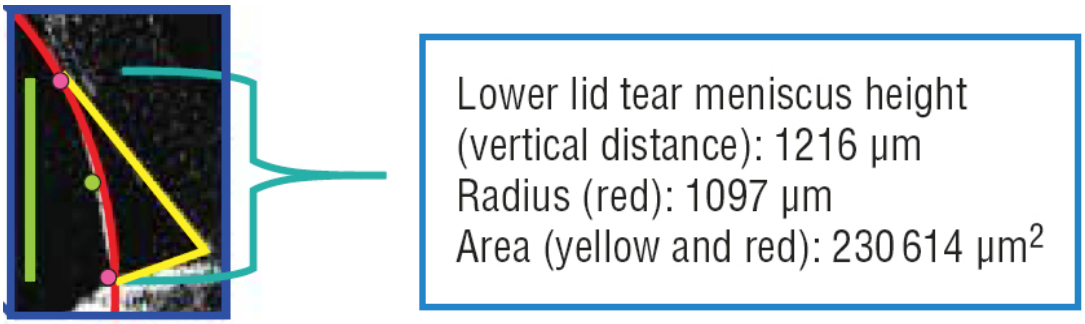


Figure 2.29: Optical coherence tomography (OCT) image of the lower tear meniscus to yield the measurement of cross-sectional area (from Wang et al. 2008)[177].

2.2.4. Tear Meniscus Volume

The measurements of tear meniscus height (TMH), tear meniscus radius (TMR) and the calculation of the cross-sectional area (TMA) are limited to one or, in the case of the area, to two dimensions. Since the meniscus is spread along the eyelid margins, the length of the lid is used to calculate the tear meniscus volume (TMV). As the eyelids are curved, the eyelid length measured on an image must be adjusted by a multiplication factor of 1.294, according to Tiffany et al.[241]. Thus the volume of the meniscus is estimated: TMV = lid length in mm x meniscus area in mm^2 x 1.294. Because the tear meniscus height seems not to be equal across the eyelid, it is suggested to use a factor of 3/4 to calculate the tear volume in the tear menisci[177, 185]. Total tear volume on the ocular surface is the sum of the tear meniscus volume and the pre-ocular tear film volume (TFV). This can be estimated as, suggested by Johnson and Murphy[163], TFV = tear film thickness in mm x exposed ocular surface area in mm^2 x 1.294.

In a group of asymptomatic non-lens wearers, a baseline of tear meniscus volume of 0.54 µL for the superior and of 0.71 µL for the inferior menisci was determined, which was significantly higher than that of soft contact lens wearers with self-reported dryness[173].

2.2.5. Tear Meniscus Regularity

Holly and Lemp[39] reported that a scanty or discontinuous inferior tear meniscus was indicative of an aqueous tear deficiency or lipid abnormality. Taylor[242] described the inferior tear meniscus as “intact“, “not intact temporally“ or “not intact“, and found

the marginal tear strip continuity to be a method of assessing the adequacy of the tear film. Guillon[243] reported that the reservoir may be interrupted and that this is one sign of potential dry eye symptoms. A subjective classification of tear meniscus profile was suggested by Khurana et al.[244] and modified by Garcia-Resua et al.[22] (Table 2.10). Grades 1 and 2 represent a healthy meniscus, whereas grades 3 and 4 represent an abnormal meniscus.

Grade	Group	Description
1	Intact	Meniscus of variable height and regular shape. Absence of debris.
2	Slightly diminished	Regular, but less visible. Absence of debris.
3	Markedly diminished or discontinuous	Diminished meniscus of irregular shape. Presence of debris.
4	Absent	Invisible meniscus.

Table 2.10: Subjective classification of the tear meniscus [22, 244]

In a group of asymptomatic non-contact lens wearers, TMH in the centre of the lower lid was significantly greater than that found in the nasal and temporal areas 3 mm from the nasal and temporal canthi[185]. In contrast, Garcia-Resua et al.[22] reported higher values of the TMH nasally and temporally at the very edge of the limbus. These differences may be explained by the different techniques, graticule technique versus optical coherence tomography, and the different locations of the measurement in the two studies. Jones at al.[185] measured the TMH adjacent to the bulbar conjunctiva, while in the study of Garcia-Resua et al.[22] TMH was measured adjacent to the limbus.

One hypothesis is that gravity forces a pool of tears to form at the centre of the eye lid[185], while others[22] hypothesise that tear fluid surface tension may explain the higher values of nasal and temporal TMH. Harrison et al.[100] showed no significant thinning of the inferior tear meniscus at the limbus compared to the central cornea, but a superior tear meniscus that thinned at the limbus in dry eye and control subjects. They also observed the movement of the tears across both menisci by instilling fluorescein close to the location of the lacrimal gland orifice. While the fluorescein moved rapidly across the lower lid towards the punctum (3 sec.), tear flow was much slower along the upper lid margin (over 35 sec.).

2.3. Correlation between Tear Meniscus, Symptoms and Dry Eye Tests

2.3.1. Symptoms and Tear Meniscus

Dry eye is best characterised by symptoms reported by the patient[5, 245, 246]. Hence, before or after a clinical examination of the tear film, the presence and nature of the symptoms needs to be ascertained and monitored. Many different dry eye questionnaires are in use to screen for the diagnosis of dry eye, or to access the effects of treatments, or to grade disease severity. The questionnaires therefore differ in the amount of questions and the purpose they were designed for.

The *McMonnies questionnaire* consists of 15 questions and is used to screen for the possibility of dry eye disease. The answers to the questions are weighted and an index scale of 0-45 is used to calculate suspicion of dry eye[38, 247]. The *Dry Eye Questionnaire (DEQ)* was developed to assess ocular surface symptoms in mild to moderate dry eye patients[248]. The DEQ includes 23 questions and has categorical scales to measure the prevalence, frequency, diurnal severity, and intrusiveness of common ocular surface symptoms. Ocular symptoms are assessed including discomfort, dryness, visual changes, soreness and irritation, grittiness and scratchiness, foreign body sensation, burning and stinging, light sensitivity, and itching[249]. The response of contact lens wearers to a dry eye survey is the issue of the *Contact Lens DEQ,* which consists of 13 questions and is designed for screening dry eye symptoms in contact lens wearers[9]. A much shorter and simpler questionnaire is the *Subjective Evaluation of Symptoms of Dryness (SESOD)* questionnaire. It consists of only three questions that are based on the frequency and

presence or absence of symptoms, and whether the symptoms interfere with daily activities[250]. *The National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25)* is a vision-targeted measure of health-related quality of life, consisting of 25 questions that produces an overall visual function score[251]. *The Ocular Surface Disease Index (OSDI)* is a 12 item questionnaire to measure the severity of dry eye disease and queried symptoms, functional problems and environmental triggers over the previous week and can be used as an end point in clinical trials[252]. *The Ocular Comfort Index (OCI)* contains 8 questions that are further separate in two parts: (1) the frequency and (2) intensity of symptoms. The focus of the OCI is the discomfort associated with ocular surface disease and was designed with Rasch analysis to produce estimates on a linear interval scale[253].

Mainstone et al.[189] reported a weak correlation between tear meniscus height and symptoms. In this study, the McMonnies dry eye survey score showed a poor correlation with tear meniscus radius, width and cross-sectional area, and a significant, but weak, negative correlation with the tear meniscus height ($r=-0.392$; $p=0.035$). TMH values were not significantly higher in asymptomatic subjects categorised by the Allergan Subjective Evaluation of symptoms of dryness (SESOD) questionnaire[156]. This lack of association between signs and symptoms was confirmed by Nichols et al.[16]., who found no correlation between TMH and symptoms using the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25). They also used a dry eye symptoms survey on dryness, grittiness, ocular fatigue, redness and soreness, but found no correlations between these symptoms and TMH. In a study by Bitton et al.[155], clinical symptoms, such as dryness and

discomfort, described by subjective 0-100 scales, were at their maximum upon eye opening, when, in contrast, TMH is at its peak.

Using the OSDI, Pult et al.[198] reported a moderate negative correlation (r=-0.450; p=0.001) to TMH measured by a slit-lamp microscope and a graticule at the centre of the lower lid, while also using the OSDI and the image capture technique of the Keratograph (Oculus, Wetzlar, Germany) to measure TMH. Finis et al.[254] reported only a weak negative correlation (r=−0.1374, p=0.0226) and Best et al.[212] noted no correlation (r=0.002, p=0.987). Furthermore, in a recent study using OCT to measure TMH and TMA no significant correlations to the OSDI score were found[229].

In conclusion, there seems to be no or only a moderate correlation between the symptoms of dryness, as described by the patient, and the volume of tear fluid in the meniscus, as measured by height and radius. This dilemma is not surprising given the lack of association between signs and symptoms in patients with dry eye disease described for many clinical tests[16-18]. It might also be attributed to the technique used to measure the tear meniscus - typically only tear meniscus height was measured; tear meniscus radius was observed in one study, but this was done with the slit-lamp in cross-section and fluorescein was applied, so the test was invasive. In none of the studies the non-invasive meniscometry was used to measure tear meniscus radius.

	Mc Monnies	SESOD	Subjective Scales 0-100	NEI-VFQ-25	Symptoms Survey[16]	OSDI	
Tear Meniscus Height	**Yes**[189]	**No**[156]	**No**[155]	**No**[16]	**No**[16]	**Yes** 198, 228, 254, 255	**No** 212, 229, 256
Tear Meniscus Radius	**No**[189]						

Table 2.11: Correlations between tear meniscus and symptoms.

2.3.2. Dry Eye Tests and Tear Meniscus

The measurement of tear volume by observing the tear meniscus is one aspect in the diagnosis of dry eye disease, but several other clinical tests are available to investigate other aspects of the tear film. Some of them are believed to assess the quantity and/or quality of tear film, while others are an indicator for the irritation of the ocular surface caused by dryness.

2.3.2.1. Schirmer Test

The Schirmer and Phenol Red Thread tests are believed to evaluate aqueous tear production or volume. The Schirmer Test has been available for over 100 years[257, 258]. It uses a strip of filter paper that is bent at one end and then inserted into the temporal part of the lower conjunctival sac (Figure 2.30). The strip is 35 mm long and 5 mm wide, and marked with a millimetre scale. After inserting the dry strip for 5 minutes, the amount of moisture is measured on a mm scale[258]. Less than 5 mm of moisture is accepted as a sign of pathological dry eye, between 5 and 10 mm is a borderline dry eye, while more than 10 mm represents a normal secretion[259]. The Schirmer Test is widely used to assess aqueous tear production, but the usefulness of

the test is debated. It is believed that the paper strip causes reflex lacrimation and therefore the test may not provide an estimation of the normal tear flow[62]. The long testing time, the discomfort caused by the strip, the risk of injuring the cornea and large variations in test results are further disadvantages of the test[259-262]. To reduce discomfort and reflex tearing, the test can be performed after the instillation of a local anaesthetic[263], but even with the use of local anaesthesia, the test is poor at measuring basal secretion rate[264].

Figure 2.30: Schirmer test with excessive reflex tear secretion.

No correlation between tear meniscus height or radius and Schirmer test with or without anaesthesia has been found in most studies[19, 30, 34, 187, 199]. This may indicate that the Schirmer test and tear meniscus evaluation measure two different aspects of the tear film, even though both are believed to be an indicator of tear volume. The invasive nature of the Schirmer test makes it more likely to measure tear flow under stimulation, while the less invasive tear meniscus method can quantify the real tear volume on the ocular surface. This was seen when using the Schirmer test with anaesthesia, when a weak correlation ($r=0.189$, $p=0.001$) and a moderate correlation ($r=0.525$, $p<0.0001$) were found in two studies using OCT to measure TMH[228, 265]. However, a recent study using a Schirmer test without anaesthesia and a swept-source OCT also reported a moderate correlation ($r=0.547$, $p=0.0038$)[231].

2.3.2.2. Phenol Red Thread Test

The Phenol Red Thread test (PRT) uses a fine cotton thread impregnated with the pH-reactive dye phenolsulfophthalein, which turns the thread yellow in air. The thread is looped over the lower lid margin in a manner similar to the Schirmer test (Figure 2.31) and left in place for 15 seconds. As a result of a tear-induced shift in pH, the yellow thread turns red when it is wetted by the tears. The further the passage of redness down the thread, the greater the tear volume. If there is less than 10 mm wetting in 15 secs then dry eye is assumed[260] The first cotton thread test was introduced by Kurihashi et al.[266] in 1975 and modified by Hamano et al.[260], who used a thread impregnated with phenol red. Compared to the Schirmer Test, the Phenol Red Thread Test causes significantly less stimulation of reflex tear production[260], and is believed to offer the ability to measure the basal secretion rate[267].

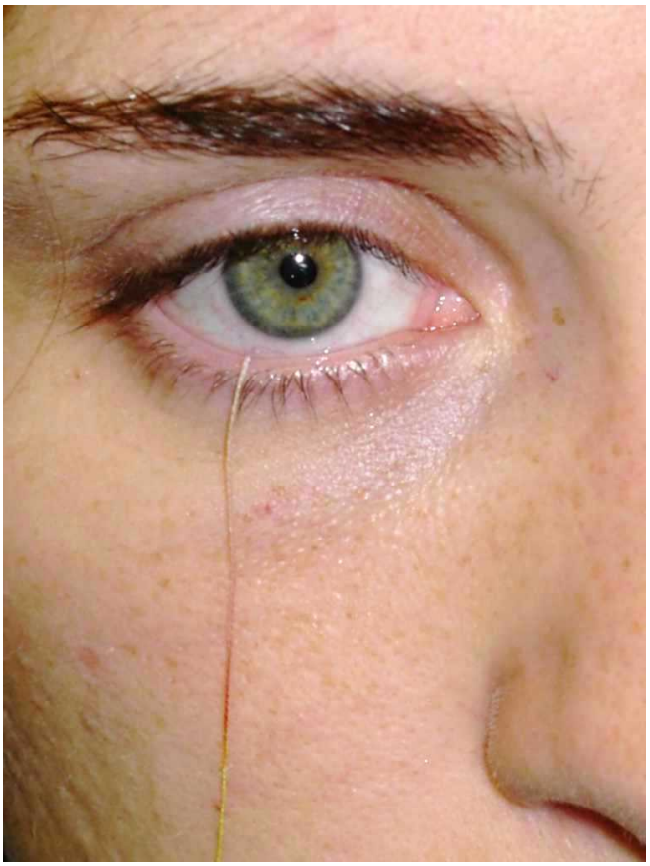


Figure 2.31: Phenol red thread test showing location of thread when in use.

However, there are reports in the literature that found no evidence that the phenol red thread test is a measure of tear production or volume, but rather describes the absorption characteristics of the thread[194]. Furthermore, Tomlinson et al.[194] reported no correlation between the phenol red thread test and tear meniscus height in a group of asymptomatic subjects, which was confirmed in a group dry eye patients[199]. In contrast, a strong correlation ($r=0.699$, $p=0.0001$) between the phenol red thread test and TMH was found by Mainstone et al.[189] and a modest correlation ($r=0.391$, $p<0.001$) was noted by Miller et al.[20], ($r=0.375$, $p<0.001$) Pult et al.[198] and ($r=0.463$, $p<0.001$) Best et al.[212] . A moderate correlation ($r=0.465$, $p=0.0096$) was also reported between the phenol red thread test and TMR[189]. These different findings

may be caused by the different subject groups and the methods of evaluating the tear meniscus and applying the PRT. Mainstone et al.[189] had a group of healthy and a group of dry eye patients that were separated by rose Bengal staining and PRT. The TMH was assessed in side view with fluorescein and PRT measures were taken with subjects keeping their eyes closed. In the study by Tomlinson et al.[194], the tear meniscus of the group of asymptomatic subjects was assessed in front view without fluorescein using an optical pachymeter and PRT were taken on the open eye in primary gaze. Miller et al.[20] compared non-lens wearers and contact lens wearers, both without dry eyes, using a graticule in front view to evaluate TMH and inserted the PRT into the open eye. Nichols et al.[199] found no correlation between PRT and TMH. In their study, tear meniscus height measurements were made using the variable beam height on the slit-lamp. There was also no correlation between the Phenol Red Thread Test and TMR in two studies by Yokoi et al. [34, 268]. Thus, like the Schirmer test, the Phenol Red Thread test seems to measure something different to the tear volume determined by tear meniscus evaluation.

2.3.2.3. Tear Film Stability

Tear film stability can be evaluated invasively by fluorescein break-up time (BUT) and non-invasively by projecting a grid or other pattern onto the tear film (NIBUT). The time interval following a complete blink to the first occurrence of breaks or a change in the reflected grid image is defined as the break-up time[269]. In BUT, 1 to 5 µl of non-preserved 2% sodium fluorescein is instilled onto the bulbar conjunctiva[270]. Within 10-30 seconds of instillation and after several natural blinks, the patient is asked to stare without blinking. Using cobalt blue illumination light

and a Wratten 12 yellow viewing filter on a the slit-lamp, the time between last complete blink and the first appearance of a black mark is recorded with a stopwatch (Figure 2.32). The test has been criticised as being inaccurate and irreproducible[271, 272]. The values of BUT are dependent on the volume of fluorescein solution instilled before measurement[273]. Furthermore, most slit-lamps' blue light and yellow barrier filters seem to be not optimal for fluorescein viewing and capture[206]. Peterson et al.[206] suggested that the use of a moistened floret or 1% minim seems most clinically appropriate as lower quantities and concentrations of fluorescein improve the efficiency of clinical examination.

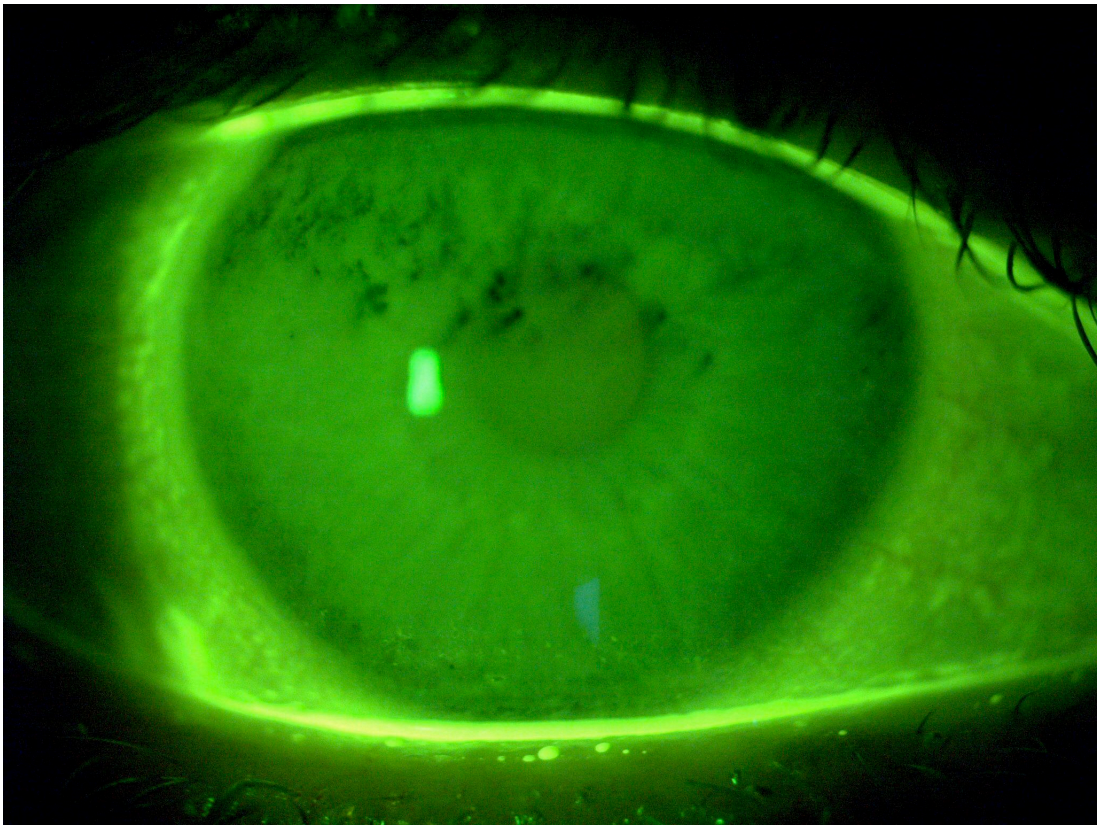


Figure 2.32: Tear film break-up made visible with fluorescein.

Korb et al.[274] developed a modification of the BUT, called the Dry Eye Test (DET), using a much smaller fluorescein strip of only 1 mm wide, compared to 5 mm of the standard fluorescein strip. The DET strip provides a significant reduction in sensation, improves measurement reliability, and enhances measurement precision, compared with a standard fluorescein strip[275]. Depending on the quantity of instilled fluorescein, the BUT cut-off values for dry eye were reported to be ≤5 secs for micro-quantities and ≤10 secs for larger quantities of fluorescein[160, 270].

While Mainstone et al.[189] reported a significant strong correlation between BUT and TMH (r=0.529, p=0.0027) and a weak correlation (r=0.345, p=0.0407) with TMR, no correlation was found in three other studies[34, 199, 205]. These discrepancies may be explained by the low reproducibility of the BUT, the different ages of the subjects, and the different techniques in measuring TM. Mainstone et al.[189] had the oldest subjects compared to the other studies and used an image capture technique, while Nichols et al.[199] used a variable height beam for TMH, Savini et al.[205] used an OCT to evaluate TMH and Yokoi et al.[34] used reflective meniscometry to measure TMR. Furthermore, Mainstone et al.[189] found a cut-off value of ≤0.35 mm, Nichols et al.[199] used <0.30 mm, while Savini et al.[205] could not define a clear cut-off value for TMH.

Using OCT to measure central TMH Czajkowski et al.[228] reported a moderate correlation (r=0.510; p<0.0001) to BUT, while using the image capture technique of the Keratograph (Oculus, Wetzlar, Germany) to measure TMH, Best et al.[212] noted no correlation to BUT.

In NIBUT the tear film break-up is measured without the influence of fluorescein by observing the distortion of a grid or other pattern projected onto the tear film. This method therefore eliminates the physical disturbance of the tear film from the instillation of fluorescein and the possibility of inducing reflex tearing[66, 272]. In contrast to BUT, the NIBUT test seems to be correlated to TMH and TMR in most of the published studies[30, 189, 198, 203, 255]. A larger tear meniscus seems to result in a longer break-up time, or, in other words, if the tear meniscus is small, the pre-ocular tear film will be unstable. Thus, tear meniscus evaluation is not only a useful test for tear film quantity, it might also be a predictor for tear film quality.

The Keratograph (Oculus Optikgeräte GmbH, Wetzlar, Germany) is the first commercially available device with software, which permits an automated, examiner independent technique for measuring NIBUT (Figure 2.33)[211]. Though, the NIBUT measurements by the Keratograph were significantly shorter than those using the Tearscope or BUT [211, 276]. Therefore, a shorter cut-off value of <2.65 sec was proposed by Hong et al.[276]. Interestingly, Best et al.[212] found no correlation between Keratograph NIBUT and TMH.

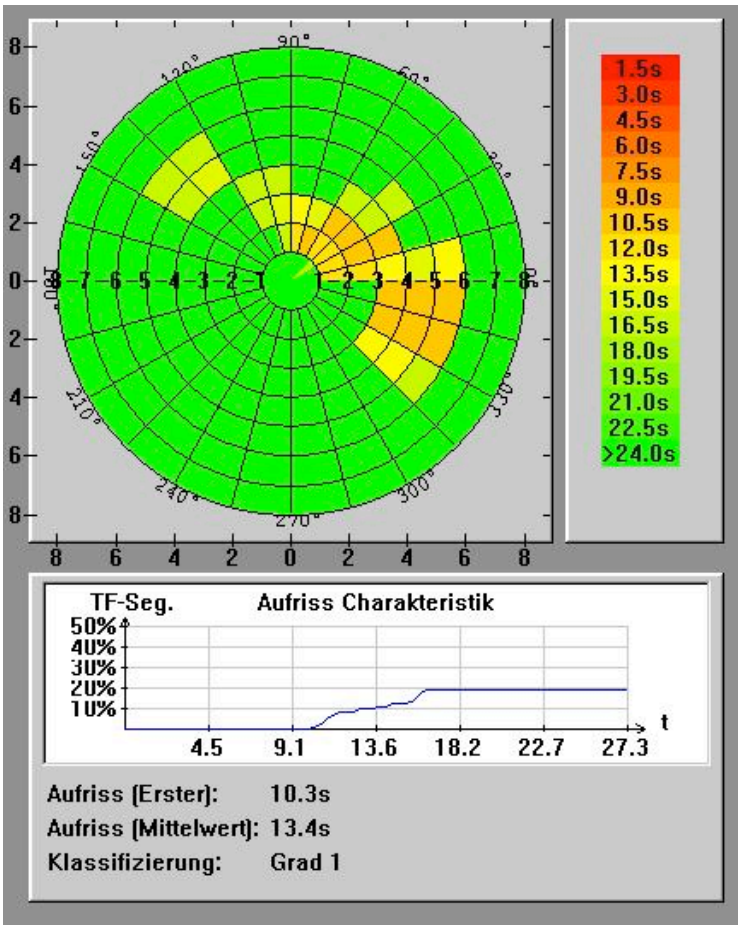
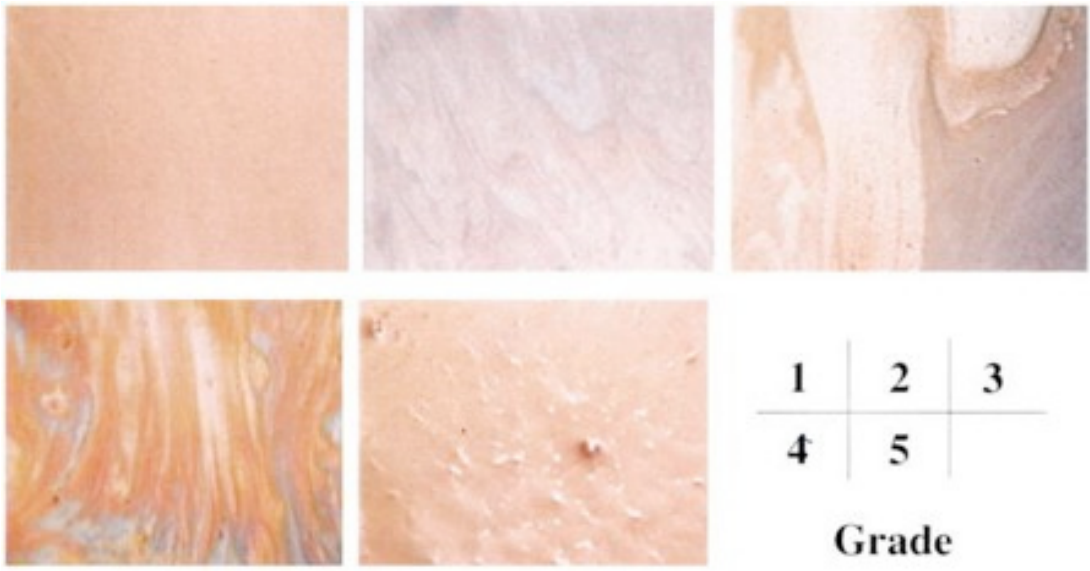


Figure 2.33: Non-invasive tear break-up time measured by the Keratograph (Oculus Optikgeräte GmbH, Wetzlar, Germany).

2.3.2.4. Lipid Layer Appearance and Spread

With specular reflection on the slit lamp, a Tearscope (Keeler, Windsor, Berks, UK) or a tear interference video-camera can observe and grade the interference pattern of the tear film lipid layer (Figure 2.34)[243]. The tear lipid layer interference patterns were significantly correlated with dry eye severity in a study by Yokoi et al.[277]. In a further study by Yokoi et al.[34], a significant inverse correlation (r=-0.52293; p=0.0125) between TMR and the grading of lipid layer interference patterns was reported. Eyes with smaller tear meniscus radii tended to show higher grades, and they argued that the lower aqueous tear volume, and the consequent reduction in the forward displacement of lid oil as the tear film was compressed during blinking, left a greater amount of oil on the lid margin for redistribution[27]. In a later study they used a video-interferometer to demonstrate that the initial velocity of the lipid layer spread after a blink decreased in proportion to the decrease of tear volume measured by meniscometry[54]. Thus they concluded that either the lipid layer spread or the tear meniscus radius may be used as an index of aqueous-deficient dry eye[54].



Grade	Appearance of the Lipid Layer
1	Uniform gray color
2	Gray color – nonuniform distribution
3	Several colors – nonuniform distribution
4	Many colors – nonuniform distribution
5	Corneal surface partially exposed

Figure 2.34: Grading system of interference lipid layer pattern[277].

No clear relationship between tear meniscus height and the lipid layer appearance was reported in a study conducted by Patel et al.[128]. In contrast to the other studies, they measured tear meniscus height with a graticule and used a Tearscope with a different grading scale. With this technique they found a thicker lipid layer in patients with a higher tear meniscus, but warned that this might be due to the uneven age distribution in their study groups. Craig et al.[278] applied a liposomal spray to one eye and found that, while the lipid layer grade was increased significantly afterwards, TMH did not alter significantly.

In modern techniques, a video-interferometer (DR-1; Kowa, Tokyo, Japan) or the placido disc of a corneal topographer (Keratograph 5M, Oculus Optikgeräte GmbH, Wetzlar, Germany) (Figure 2.35) have been used to visualise the tear film lipid layer interference pattern.

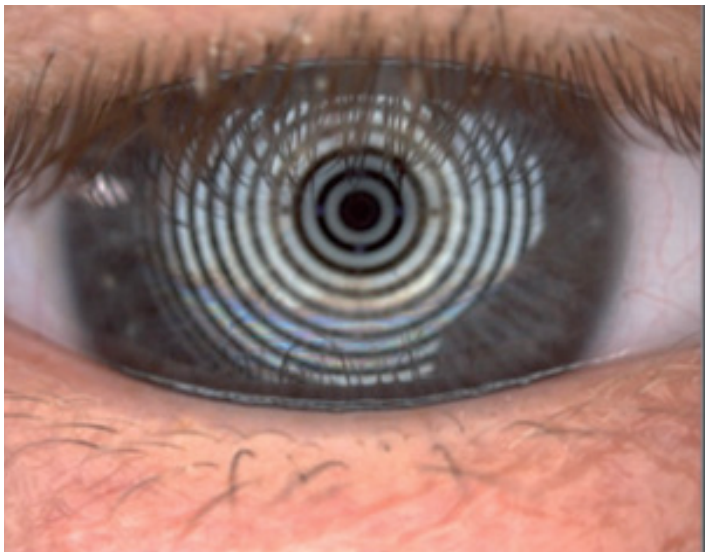


Figure 2.35: Projection of a placido disc of a corneal topographer (Keratograph 5M, Oculus Optikgeräte GmbH, Wetzlar, Germany) to visualise tear film lipid layer pattern.

However, with both techniques a subjective grading of the lipid layer technique is still necessary. An objective quantitative measurement of lipid layer thickness is a new promising method that can be performed with the LipiView Interferometer [279-281](TearScience, Morrisville, North Carolina, USA) (Figure 2.36).

Figure 2.36: LipiView® (TearScience, Morrisville, North Carolina) for objective, quantitative measurement of the tear film lipid layer thickness.

2.3.2.5. Meibomian Gland Evaluation

Meibomian Gland Dysfunction (MGD) is believed to be the most common cause of evaporative dry eye, which may also have some association with aqueous-deficient dry eye[110, 282, 283]. The MGD Workshop group has proposed the following definition of MGD: "Meibomian gland dysfunction (MGD) is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. This may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease[284]."

To assess the severity of MGD it is important to evaluate the morphology and function of the Meibomian Glands[285]. MG status and function can be evaluated by

observing the lid margin with a slit-lamp[285, 286], by analysing the inference pattern of the lipid layer[279, 280], by testing the MG expressibility[287], and by observing the MG morphology with meibography[285, 288].

The severity of the MG findings can be categorised by existing grading scales (Figures 2.37 and 2.38).

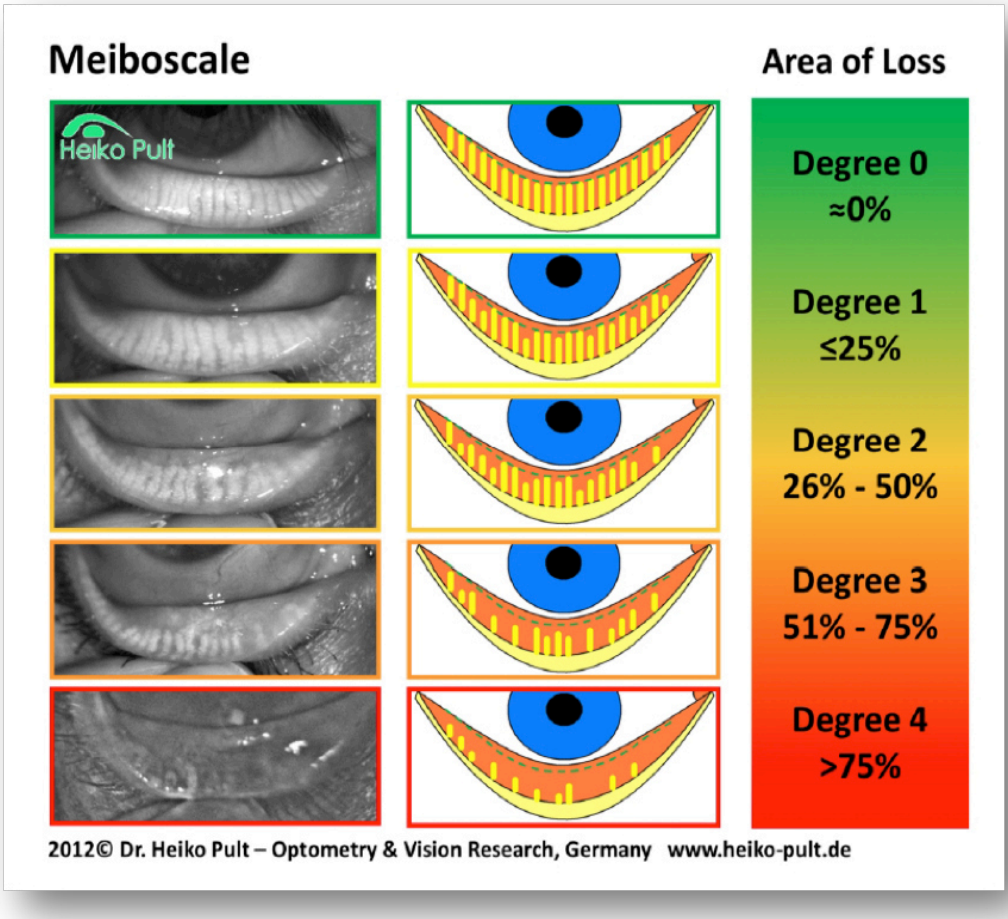


Figure 2.37: Grading of MGD according to meibography findings (from Pult and Riede-Pult [289].

Classification and Grading System			Grade	
Eyelid Margin				
Thickness (measured posterior margin to the posterior lash line)			0–5	☐
Rounding of posterior margin			0/1	☐
Irregularity; notching of margin			0/1	☐
Vascularity of lid margin: telangiectasia			0/1	☐
Lash loss			0/1	☐
Trichiasis or distichiasis (state)			0/1	☐
Malapposition			0/1	☐
Anterior blepharitis			0/1	☐
Mucocutaneous junction				
Anteroplacement			0–3	☐
Retroplacement			0–3	☐
Ridging			0/1	☐
Mucosal Absorption			0/1	☐
Orifices				
	Upper Lid	Lower Lid		
Number present (central 1 cm)	_____	_____		
Number patent (central 1 cm)	_____	_____		
Pouting or plugging			0/1	☐
Narrowing			0/1	☐
Loss of cuffing definition			0/1	☐
Opaque/scarred			0/1	☐
Vascular invasion			0/1	☐
Retroplacement			0–3	☐
Other: (state)				
Main Duct				
Exposure (1 = <1 mm exposed; 2 = ≥1–2 mm; 3 = ≥2 mm)			0–3	☐
Cystoid dilatation			0–3	☐
Acini				
Visibility (1 = clusters; 2 = yellow stripes; 3 = not visible)			0–3	☐
Concretions (1 = deep; 2 = subepithelial; 3 = extruding)			0–3	☐
Chalazia			0–3	☐
Expressed Secretions				
Foam			0/1	☐
Volume: (score the diameter of the largest pool expressed)			mm	☐
Quality: (0 = clear; 1 = cloudy; 2 = granular; 3 toothpaste)			0–3	☐
Expressibility: (1 = light; 2 = moderate; 3 = heavy pressure)			0–3	☐

Grading is performed in the grade range listed, and in cases of 0/1, 0 = no/normal, and 1 = yes/abnormal. Reprinted in modified form with permission from Bron AJ, Benjamin L, Snibson GR. Meibomian gland disease: classification and grading of lid changes. *Eye.* 1991;5:395–411; and Foulks G, Bron AJ. A clinical description of meibomian gland dysfunction. *Ocul Surf.* 2003;1:107–126.

Figure 2.38: Grading of MGD According to Clinical Features and Gland Expression (from Tomlinson et al. 2011)[286].

There are no reports in the literature that describe any relationship between the severity of MGD and TMH. However, in a study by Cuevas et al. they found a significant increase in TMH after medical treatment of patients with MGD[290].

2.3.2.6. Tear Film Thickness

In the literature, the value for the thickness of the precorneal tear film (PCTF) varies between 2.7 μm[291] and up to 46 μm[292], depending on the measuring technique used. The thickness of the PCTF can be measured by interferometry, fluorometry, optical pachymetry, confocal microscopy and optical coherence tomography. Measurement with the interferometer gives the thinnest, and with the confocal microscope the thickest, PCTF measured[59].

A mathematical relationship, where the thickness of the precorneal tear film is proportional to the tear meniscus radius, was proposed by Wong et al.[293] and applied by Creech at al.[232] (Figure 2.39).

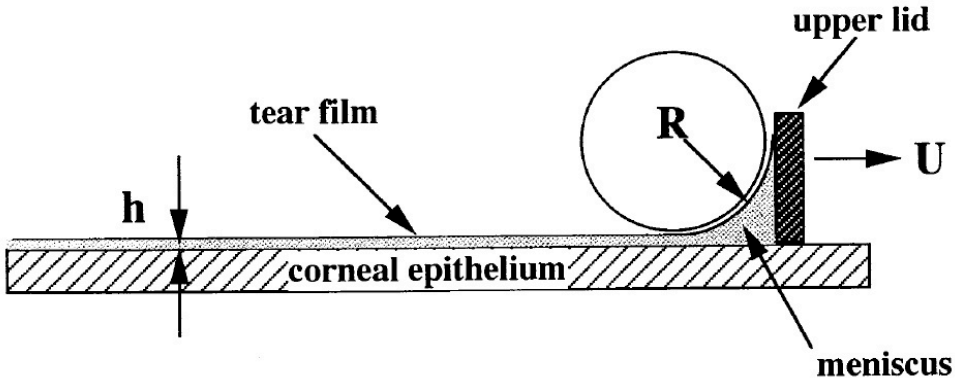


Figure 2.39: Model of relationship between tear meniscus radius and tear film thickness (from Creech et al.1998)[232].

Creech et al. applied fluorescein and videotaped the TMR in profile and calculated a tear film thickness of 10.4 µm in a non-lens group, 6.5 µm in hydrogel lens wearers and 5.8 µm in RGP lens wearers[232]. Yokoi et al.[54] continued this concept and found TMR to be proportional to tear volume[29], and that TMR correlates to the velocity of the tear film lipid layer, suggesting that a low TMR may imply a low tear film thickness that is responsible for slow lipid layer spreading. Using an interference thickness measurement device, Hosaka et al.[294] found a significant correlation between the estimated tear film thickness and TMH ($r= 0.42$; $p= 0.006$). However, no correlation between any tear meniscus variable and tear film thickness, both determined simultaneous by an OCT, was found by Wang et al[32].

Interestingly, Wang et al.[238] reported in another study, using the same instrumentation, that, while at baseline there was no correlation, after the instillation of artificial tears strong correlations between tear film thickness and inferior tear meniscus height and area were found. They attributed this difference to the increased fluid volume being pulled by gravity towards the lower lid and pointed out that further studies should be performed to compare the influence of different artificial tears to the tear meniscus.

2.3.2.7. Turnover Rate

Tear turnover rate (TTR) is defined as the rate of change in tear volume over a set time period, and can be demonstrated by assessing the percentage decrease of fluorescein concentration in the tears per minute after fluorescein instillation[80], a method called the fluorescein clearance test (FCT). A standardised volume and

concentration of sodium fluorescein is instilled into the inferior conjunctival cul-de-sac and tear turnover is determined by the persistence of fluorescein after a specific time. The remaining fluorescein can be collected with a Schirmer strip or a glass capillary from the inferior tear meniscus. The fluorescein concentration of the collected sample can be assessed with a fluorometer or a visual scale[295, 296].

Tear turnover can also be assessed *in vivo* with an automated scanning fluorophotometer (Fluorotron Master, OcuMetrics, Mountain View, CA, USA) (Figure 2.40)[297, 298]. Nelson et al.[298] found tear turnover to be 42% lower in patients with keratitis sicca and Sobara et al.[299] reported a significantly reduced tear turnover in patients with symptomatic dry eye. Tear turnover showed no correlation to Schirmer test or phenol red thread test[194, 298]. Using the automated scanning fluorometer and a slit-lamp to measure tear volume, no correlation between tear turnover rate and TMH was found by Tomlinson et al.[194]. However, Savini et al.[205] showed a correlation ($r=0.4912$, $p=0.0006$) between TMH and fluorescein clearance, but they used a Schirmer strip to collect the remaining fluorescein and an OCT to measure TMH, which may explain the different findings.

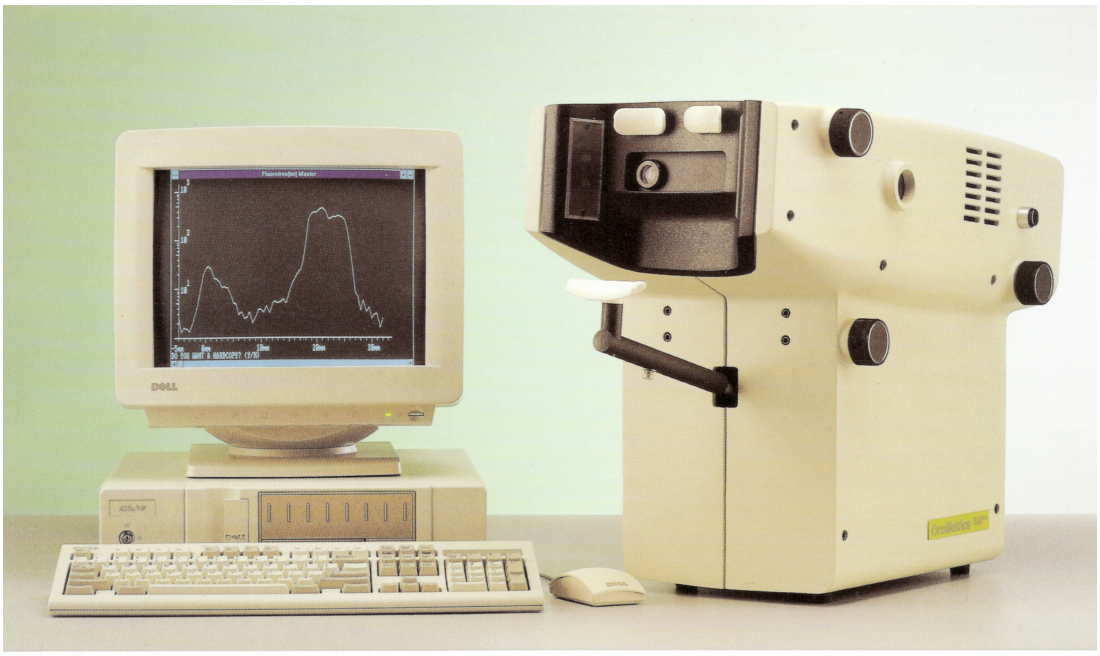


Figure 2.40: The FM-2 Fluorotron Master (OcuMetrics Inc, Mountain View, CA, USA).

A new method for evaluation of early phase tear clearance by anterior segment optical coherence tomography was recently introduced by Zheng et al.[300]. Since with this technique the tear clearance is observed with an OCT no fluorescein has to be applied.

2.3.2.8. Osmolarity

Osmolarity is the measure of solute concentration, such as sodium and potassium, in the aqueous of the tear film and is expressed by the unit mOsm/L. The loss of tear fluid by increased evaporation or decreased production of tear fluid may result in hyperosmolarity. According to the definition of dry eye by the Dry Eye Workshop, tear hyperosmolarity may be regarded as the signature feature that characterises the condition of "ocular surface dryness"[5, 270]. Therefore, some authors describe the

measurement of tear film osmolarity as the gold standard in dry eye diagnosis[301, 302]. Cut-off values between 312 and 318 mOsm/L have been proposed in different studies[12, 71, 301]. In the past, this test required the collection of a tear specimen by dipping the end of a microlitre glass capillary tube into the lower tear meniscus and a special technical instrument called a Clifton Freezing Point Nano-Osmometer was used to analyse the samples. It is believed that the general utility of osmolarity measurement has been hindered by the need for expert technical support and therefore was limited to a small number of specialised laboratories[270]. In 2008, a commercially available device for eye care practitioners, with a lab-on-a-chip technology to measure tear osmolarity, was introduced to the market (Figure 2.41). This instrument was found to be precise and accurate with a sensitivity of 87% and a specificity of 81%[303, 304].

Figure 2.41: TearLab™ instrument to measure tear osmolarity (TearLab Corporation, San Diego, CA, USA).

In a group of patients with nasolacrimal duct obstruction, Stahl et al. found an increase in TMH, compared to a control group, and a decrease in TMH after successful dacryocystorhinostomy[188]. However, tear osmolality was similar in both the normal group and the watery eye group, and was unaffected by the surgery. They concluded that to maintain normal tear osmolality in a patient with nasolacrimal duct obstruction, tear production must be reduced, to permit the concentration of solute particles to remain constant[188]. Likewise, no relationships between tear osmolarities and tear meniscus volumes were observed by Li et al.[305].

It is suggested, that the osmolarity in the tear film is higher than that in the menisci, which might underestimate the osmolarity in DED if the sample is taken from the menisci[83].

2.3.2.9. Conjunctival Redness

Conjunctival hyperaemia is the result of an increase in the diameter of blood vessels and can be a response to mechanical, toxic or allergic irritation, or to an inflammation of the anterior eye. Furthermore, dryness or low oxygen supply caused by contact lens wear is able to induce a conjunctival hyperaemia[306, 307]. Hyperaemia of the conjunctiva is assessed by a slit-lamp with diffuse white illumination and a magnification of about 10x or 12x. The redness can be graded with several grading scales like the CCLRU[308]-Grading scale (Figure 2.42) or the Efron[309]-Grading Scale. To improve the sensitivity of the scale, an interpolation of the five unit scales to 0.1 increments is recommended[310]. Furthermore, an objective image analysis of bulbar

hyperaemia, introduced by Peterson and Wolffsohn[311, 312], was found to be 16x more reliable than subjective analysis.

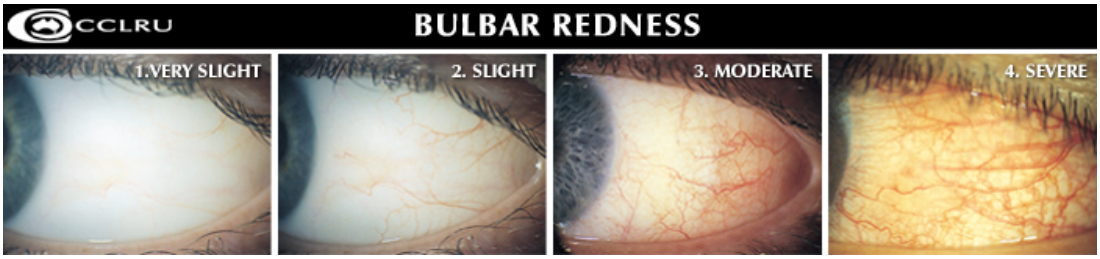


Figure 2.42: Bulbar conjunctival hyperaemia, demonstrated by the CCLRU grading scale (Johnson & Johnson Vision Care, Inc., Jacksonville, Florida, USA)

Bitton et al. showed that ocular redness increases upon waking, but quickly returns to baseline after an hour of eye opening, with no difference between a dry eye group and a non-dry eye group[155]. They argued that this redness is caused by some degree of subclinical inflammation and hypoxia that occurs overnight in the closed eye environment. Furthermore, they found no correlation between bulbar redness of the eye and tear meniscus height. These findings were confirmed by Pult et al.[198] and Best at al.[212], who found no correlation between TMH and bulbar or limbal redness[255].

2.3.2.10. Ocular Surface Staining

In optometric practice a variety of staining agents is available to evaluate the ocular surface. Besides the frequently used sodium fluorescein, other dyes like rose Bengal and lissamine green are employed to study the tear film and the status of the cornea and conjunctiva. Fluorescein diffuses into intercellular spaces and therefore stains

disruptions of cell to cell junctions or epithelial cell dropout[313]. Fluorescein neither stains healthy cells nor is it able to detect dead or degenerated cells[314]. Because fluorescein may stain the matrix of soft contact lenses, high molecular weight fluorescein (fluorexon) can be used when fitting soft contact lenses[315].

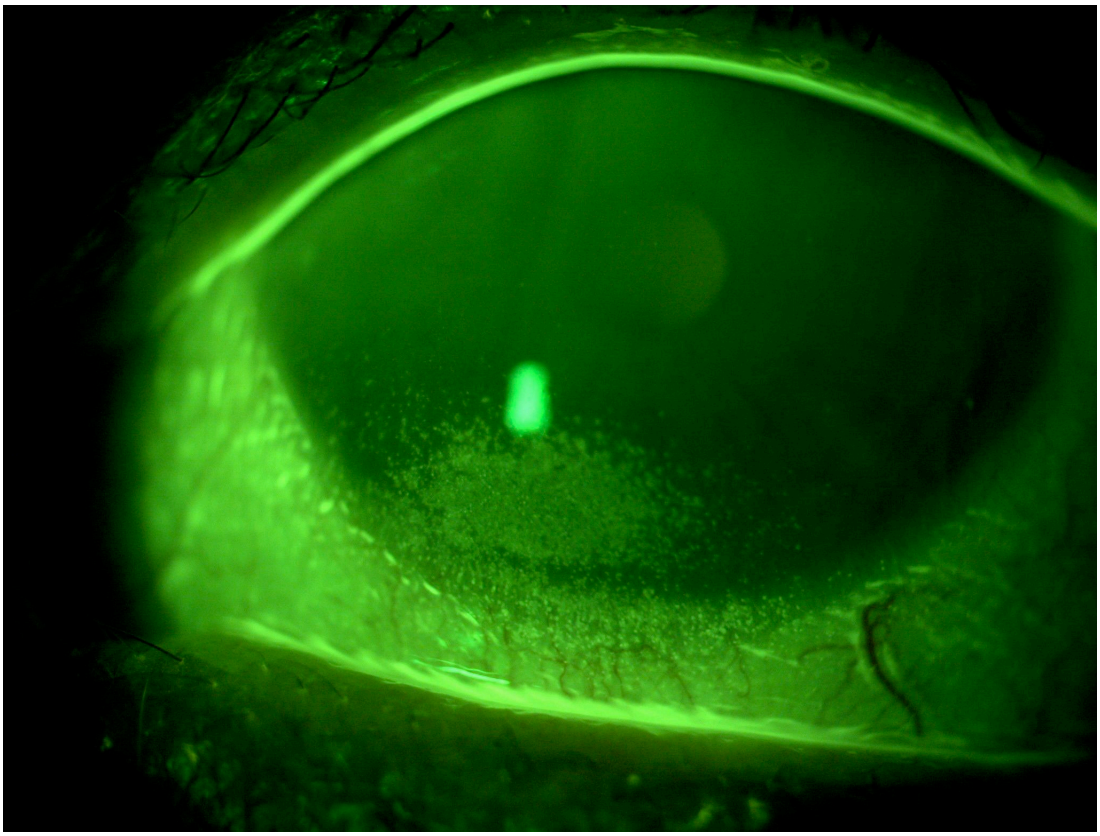


Figure 2.43: Desiccation of the inferior cornea stained with fluorescein.

Rose Bengal is a derivate of fluorescein and, in contrast, stains healthy and dead or degenerated cells[313]. When the corneal or conjunctival epithelium is covered by an intact preocular tear film, the mucins block the access of the dye to the cells[316]. The use of rose Bengal, however, appears to be decreasing[317]. This is likely to be the result of the toxicity and the stinging when the dye is instilled[314, 316-318].

Like rose Bengal, lissamine green stains dead or degenerated cells, but it causes less stinging, so it is suggested as a substitute[318-320]. Superior to rose Bengal, lissamine green does not stain healthy cells and the dye is not blocked by mucins[314]. Nevertheless, lissamine green and rose Bengal show similar staining patterns, measured by the van Bijsterveld scale, in patients with mild to moderate dry eye syndrome[314, 321].

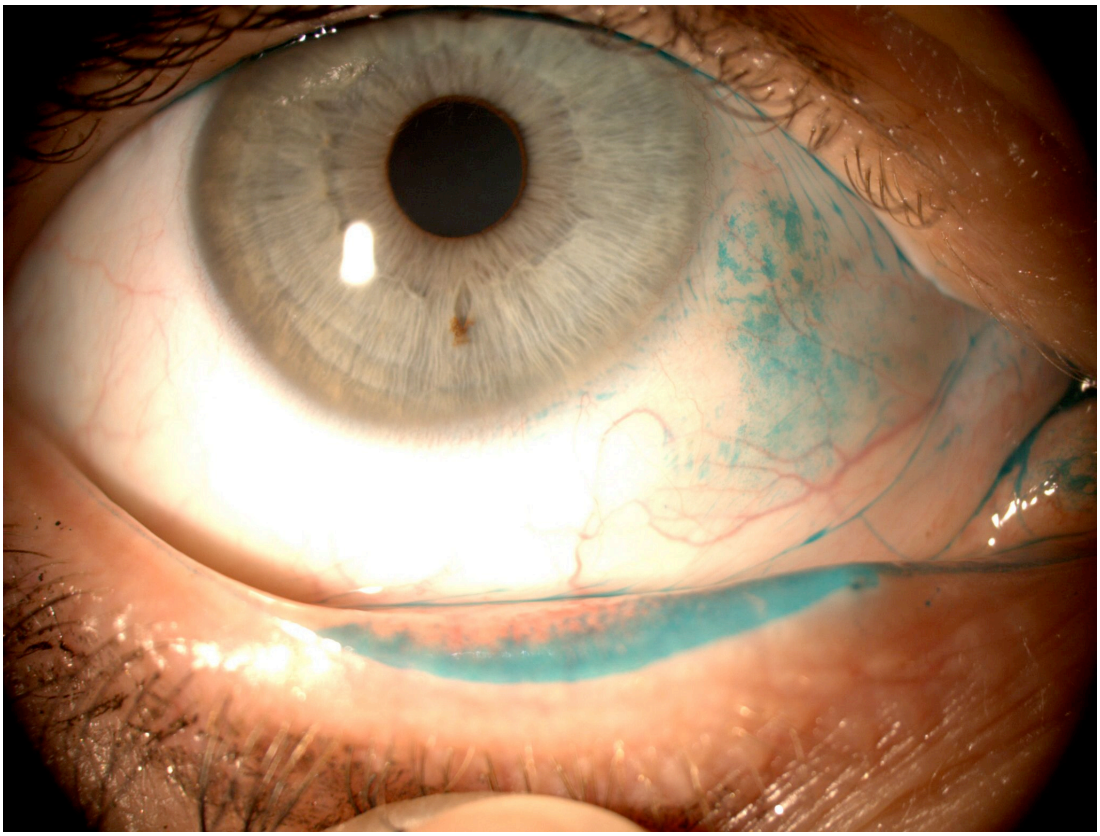


Figure 2.44: Lissamine green staining of the bulbar and tarsal conjunctiva caused by dryness.

While fluorescein seems to be the most effective dye for corneal staining, lissamine green or rose Bengal are more effective for conjunctival staining[317]. Korb et al.[317]

suggested a mixture of 2% fluorescein and 1% lissamine green for excellent simultaneous corneal and conjunctival staining without adverse sensation. The location and the extent of ocular surface staining can be graded with different grading scales. Uchiyama et al.[322] described that the presence of nasal conjunctival lissamine green staining is associated with mild dry eye, that nasal and temporal staining is associated with moderate dry eye, while the presence of nasal and temporal conjunctival and corneal staining correlates with more severe dry eye, as diagnosed with the Schirmer test.

Using the van Bijsterveld staining score[323] Mainstone et al.[189] reported a significant negative correlation between tear meniscus height measured with image capture and fluorescein staining ($r=-0.663$, $p=0.0007$) and rose Bengal staining ($r=-0.597$, $p=0.0013$). In contrast Nichols et al.[199] found no correlation between TMH and fluorescein or rose Bengal staining, but a strong relation between the phenol red thread test and both fluorescein ($r=-0.48$, $p < 0.0001$) and rose Bengal staining ($r=-0.29$, $p=0.01$), and also a strong relation between Schirmer test and fluorescein staining ($r=-0.32$, $p=0.005$).

Nichols at al. used the CLEK Schema proposed by the National Eye Institute workshop[324] to grade ocular surface staining and measured the TMH with a variable beam height on the slit-lamp. Furthermore, their selection of dry eye patient was based on the Classification of Diseases for dry eye syndrome, while Mainstone et al.[189] used the phenol red thread test and rose Bengal staining to diagnose aqueous deficient dry eye. Different findings may be attributed to differences in patient selection, tear meniscus height evaluation and grading of staining. These findings of

Nichols et al.[199] were confirmed by the study of Savini et al.[205] who also found no correlations between TMH (analysed by an OCT) and rose Bengal and fluorescein staining. Their diagnosis of aqueous tear deficiency was based on the fluorescein clearance test. They argued that rose Bengal and fluorescein staining do not occur in all patients with reduce tear secretion, but only in those cases with a more advanced stage of dry eye[205]. No correlation between fluorescein or lissamine green staining and TMH were reported by Pult et al.[255] and by Best et al.[212]

In a recently published study comparing a group of patients with MGD to a group with aqueous tear deficiency (ATD), a significant lower TMH was found in the ATD[229]. Interestingly, while in the ATD group a lower TMH was related to more corneal staining, in the MGD group a higher TMH showed more corneal staining[229]. They hypothesised that higher tear volume in eyes with MGD may prove to be a sign that potentially damaging mediators could be retained on the ocular surface and require therapies to improve tear clearance or treatment of inflammation[229]. However, in both groups no correlation between TMH and lissamine green conjunctival staining was observed[229].

2.3.2.11. Lid Parallel Conjunctival Folds (LIPCOF)

Lid-parallel conjunctival folds (LIPCOF) are folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin[325, 326] (Figure 2.45). LIPCOF are easily observable with a slit-lamp microscope and are evaluated without fluorescein. The LIPCOF evaluation is performed in the area perpendicular to the

temporal and nasal limbus, and the findings are classified using a grading scale[198, 327] (Table 2.12).

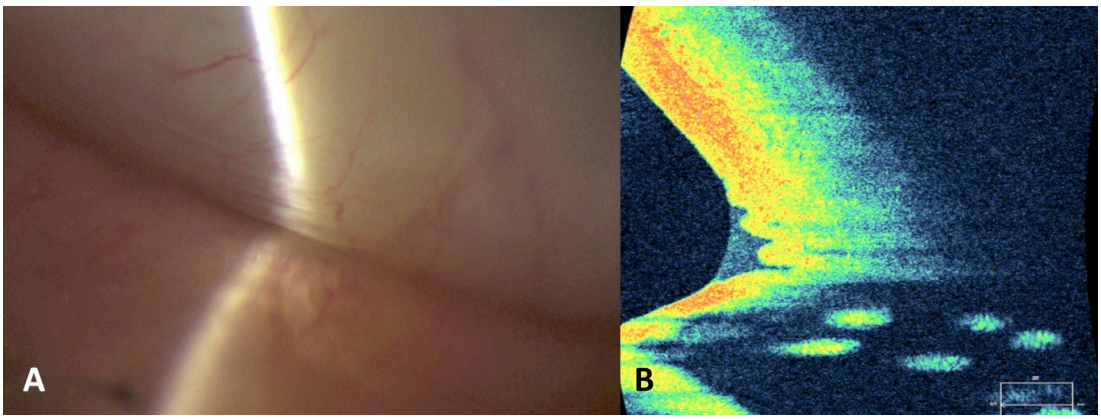


Figure 2.45: Slit-lamp image (A) and OCT image (B) of LIPCOF grade 3 at the temporal position.

Lid-parallel conjunctival folds were described as a sub-type that might represent a mild stage of conjunctivochalasis[326]. LIPCOF scores have been reported to be increased in dry eye, but they are not age-related[325, 328], while conjunctivochalasis has been defined as the redundant, loose, non-oedematous conjunctival tissue found at the lower eyelid, typically in older people[329, 330]. Conjunctivochalasis is often used to describe more prominent folds than described by LIPCOF, being around 0.08 mm height[331]. On the other hand, LIPCOF has to be differentiated from micro-folds, which are less well organised and are around three times smaller than LIPCOF[331].

LIPCOF Grade	
0	No conjunctival folds
1	One permanent and clear parallel fold
2	Two permanent and clear parallel folds, (normally <0.2 mm)
3	More than two permanent and clear parallel folds, (normally >0.2 mm)

Table 2.12: Optimised grading scale of LIPCOF[198, 327].

Like conjunctivochalasis, LIPCOFs are located in the tear meniscus area and both are assumed to interfere with the meniscus[329, 332-334]. Huang et al.[334] found that the conjunctival folds in conjunctivochalasis obliterate tears not only in the meniscus, but also in the reservoir, and they assumed that the conjunctival folds could occupy and deplete the tear reservoir in the fornix. However, in a study by Pult et al.[198], TMH, which was measured by a slit-lamp microscope and a graticule at the centre of the lower lid, showed no correlation to temporal or nasal LIPCOF grade.

2.3.2.12. Lid Wiper Epitheliopathy (LWE)

The lid wiper is defined as that portion of the marginal conjunctiva of the upper eyelid that wipes the ocular surface during blinking[335, 336]. In the dry eye patient the tear film is insufficient to lubricate the ocular surface, causing continual rubbing and therefore trauma to the lid wiper region at each blink[335, 336]. To visualise the damage of the epithelial cells at the lid wiper region, a combination of fluorescein and rose

bengal or lissamine green is used (Figure 2.46)[317]. The LWE is classified by measuring the length and the width of the stained area after lifting the patient's upper lid and the finding is graded in a four-degree scale[335].

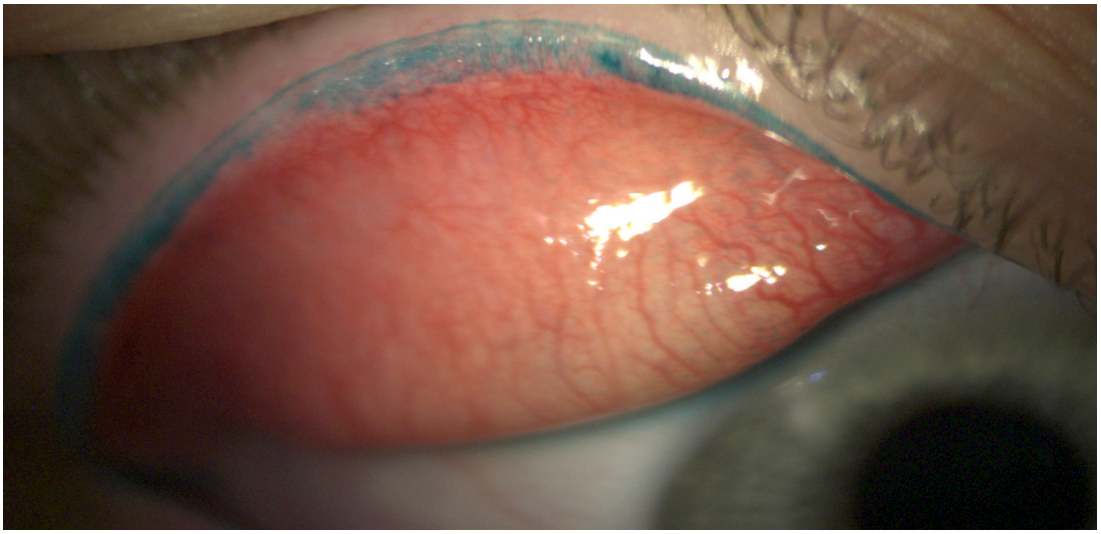


Figure 2.46: Lid-wiper epitheliopathy of the upper eyelid margin stained with lissamine green.

LWE is found in 67% to 80% of symptomatic CL wearers, but in only 13% to 32% of asymptomatic subjects[337, 338]. In diagnosed dry eye patients, LWE was detected in 18.7%[339]. A recent study also reported about a LWE-like staining at the lower eyelid margin with a significant higher prevalence (39.5%) than the upper-LWE (12.0%) in a group of non-CL wearers[340]. They assumed that the higher prevalence of lower-LWE may be caused by the continuous friction of the lower eyelid on the same region of the cornea during blinking[340].

In two other studies, no correlation was found between LWE and TMH measured at the centre of the lower lid[198, 212].

2.3.2.13. Summary

The correlations between tear meniscus height or tear meniscus radius and the different dry eye tests as described in Chapter 2.3.2 are summarized in Table 2.13:

	Phenol Red Thread Test		Schirmer Test	Schirmer Test with anesthesia		BUT		NIBUT		Lipid Layer Appearance
Tear Meniscus Height	**Yes** 20, 189, 198, 212	**No** 194, 199, 255	**No** 19, 187, 199	**Yes** 28, 228, 265	**No** 19, 30	**Yes** 189, 228	**No** 199, 205, 212	**Yes** 30, 189, 198, 203, 255	**No** 212	**No** 128
Tear Meniscus Radius	**Yes** 189	**No** 34, 268		**Yes** 28	**No** 30, 34	**Yes** 189	**No** 34	**Yes** 189		**Yes** 34, 54

	Fluorescein Staining		Rose Bengal Staining		Lissamine Green Staining	Turnover Rate		Osmolality	Conjunctival Redness
Tear Meniscus Height	**Yes** 189, 229	**No** 199, 205	**Yes** 189	**No** 199, 205	**No** 229, 255	**Yes** 205	**No** 194	**No** 188, 305	**No** 155, 198, 212, 255
Tear Meniscus Radius	**Yes** 34, 189		**Yes** 189						

	Tear Ferning	Tear Film Thickness		LIPCOF	LWE	Tear Film Debris
Tear Meniscus Height	**No** 155	**Yes** 294	**No** 32, 238	**No** 255	**No** 212, 255	**No** 189
Tear Meniscus Radius		**Yes** 232				**No** 189

Table 2.13: Summary of correlations between tear meniscus measurement and other dry eye tests.

2.4. Sensitivity and Specificity of Tear Meniscus Measurement

As mentioned in Chapter 2.2, the different methods for evaluating tear meniscus height and curvature has produced a large variety of values in healthy and unhealthy eyes. This causes difficulties in establishing cut-off values since each value is only valid for one method. As a consequence there is no universally accepted cut-off value for normal eyes in tear meniscus height measurement. Mainstone et al. used a cut-off value of 0.35 mm[189], Nichols et al. used 0.3 mm[16], Doughty et al. suggested 0.25 mm and 0.1 mm[24], Farrell et al. defined 0.18 mm[341], while Shen et al. calculated 0.164 mm as the cut-off point[28].

2.4.1. Tear Meniscus Height

Shen et al. found good dry eye diagnostic accuracies (sensitivity and specificity of 0.92 and 0.90[28]) with a cut-off value for an "abnormal" inferior tear meniscus height (ITMH) of 0.164 mm. In their study they used an OCT to measure TMH. In comparison, with an image capture system, Farrell et al. defined an arbitrary cut-off value of 0.180 mm to obtain sensitivity and specificity values of 0.73 and 0.67[341], and Mainstone et al. applied an image capture system and a cut-off value $\leq$ 0.350 mm[189] and reported a sensitivity of 0.93 and specificity of 0.67 (Table 2.14).

2.4.2. Tear Meniscus Radius of Curvature

Shen et al. also found good dry eye diagnostic accuracies (sensitivity and specificity were 0.92 and 0.87) with a cut-off value for an "abnormal" inferior tear meniscus radius (ITMR) of 0.182 mm[28]. In contrast, Mainstone et al. suggested a cut-off value ≤0.350 mm using an image capture technique, and found a sensitivity of 0.80 and specificity of 0.87 (Table 2.14)[189].

Author	Technique	TMH	TMR	TMD	TMA
Ibrahim et al.[342]	Time Domain OCT	Sensitivity 67% Specificity 81% Cut-off <0.300mm			
Czajkowski et al.[228]	Spectral Domain OCT	Sensitivity 81% Specificity 89%		Sensitivity 78% Specificity 53%	Sensitivity 86% Specificity 85%
Wang et al.[343]	AS-OCT	Sensitivity 78% Specificity 72% Cut-off <0.213mm			
Mainstone et al.[189]	Image Capture System	Sensitivity 93% Specificity 67% Cut-off <0.350mm	Sensitivity 80% Specificity 87% Cut-off <0.350mm		
Farrell et al.[341]	Image Capture System	Sensitivity 73% Specificity 67% Cut-off <0.180mm			
Shen et al.[344]	Custom made real time OCT	Sensitivity 92% Specificity 90% Cut-off <0.164mm	Sensitivity 92% Specificity 87% Cut-off <0.182mm		
Pult et al.[198]	Slit-lamp with graticule	Sensitivity 65% Specificity 65% Cut-off <0.200mm			
Bandlitz and Berke[207]	Slit-lamp with graticule	Sensitivity 56% Specificity 74% Cut-off <0.300mm			
Yokoi and Komuro[26]	Video-Meniscometry		Sensitivity 89% Specificity 78% Cut-off <0.250mm		

Table 2.14: Summary of sensitivity and specificity reported for tear meniscus measurements.

CHAPTER 3: Review Conclusions and Plan

The aetiology of dry eye is classified into evaporative dry eye (EDE) and aqueous-deficient dry eye (ADDE)[5]. Aqueous deficient dry eye is due to a failure of lacrimal tear secretion leading to a reduced tear volume[5]. The Schirmer and Phenol Red Thread tests are the classic tests that are believed to evaluate aqueous tear production or volume[20, 259]. However, the invasive nature of both tests makes it more likely to measure tear flow under stimulation and not the real tear volume. Furthermore, the results of the Schirmer Test are variable[259, 345, 346] and, in the case of the Phenol Red Thread test, no evidence was found that the test is a measure of tear production or volume, but may instead describe the absorption characteristics of the thread[194].

In contrast, the non- to minimal invasive methods of tear meniscus evaluation can quantify the real tear volume on the ocular surface[29, 31]. The tear meniscus can be analysed by its height (TMH)[22, 23, 189, 200] and curvature (TMR)[26-28, 30, 31, 163, 189]. Various methods have been used to measure the TMH, and, depending on the technique used, TMH in normals varies between 0.12 and 0.46 mm[23, 189]. Since the tears are transparent, sometimes fluorescein is used to facilitate tear meniscus height measurement. However, the differences in the TMH measuring techniques -with or without flourescein, with a graticule or variable beam height at the slit-lamp, with a pachymeter, or with image capture- may explain the huge variation and the poor repeatability of the current methods of tear meniscus height evaluation.

The TMR can be measured with image capture, optical coherence tomography or reflective meniscometry. In image capture and in OCT the meniscus is viewed in

cross-section, and TMR is assessed by determining the radius of a circle that best fits the curved anterior meniscal surface. This is very complicated and, because the outer edges of the tear meniscus are difficult to define in some cases, it is also inaccurate. With image capture, the use of fluorescein results in a more invasive and inaccurate test, while with OCT and reflective meniscometry, no dye is needed to visualise the curvature of the meniscus. Therefore, values of the inferior TMR in normals with the OCT (0.34 ± 0.23 mm) are similar to those measured with meniscometry (0.37 ± 0.15 mm). Optical coherence tomographers are still expensive and are not standard equipment in clinical practice. Meniscometry provides a clear and stable image of the tear meniscus. Therefore, the upper extremity of the meniscus can easily be detected, contrary to the tear meniscus height measurements. Meniscometry is a precise and repeatable technique and since the tear meniscus radius is correlated to the tear volume, meniscometry is an effective tool in the diagnosis of aqueous-tear deficiency[28, 29]. However, those reflective meniscometer developed are mostly prototypes and there are only three in circulation (personal communication with Prof. Anthony J. Bron). Consequently the aims of this PhD are:

(i) improve the evaluation of the tear meniscus for the clinician by developing an advanced observation device, (ii) investigate the relationship between the tear meniscus radius (TMR) and the tear meniscus height (TMH), as well as the effect of area of observation in normal and dry eye patients, and (iii) further explore the impact of tear supplements on the menisci.

To address this aims, the PhD will take the following steps:

1. Development of a new portable meniscometer that can be easily used by the clinician. Chapter 4 describes the construction, the adjustment and the calibration of such a new instrument named the portable digital meniscometer (PDM).

2. Once the instrument is developed further investigation is necessary to prove its accuracy and repeatability compared to the existing standard instrument for meniscometry (video-meniscometer). Chapter 5 evaluates the *in vitro* and *in vivo* performance of the PDM.

3. Tear meniscus radius can also be measured by optical coherence tomography. Chapter 6 compares the new PDM to the OCT technique in tear meniscus evaluation.

4. Chapter 7 investigates the ability of the PDM to measure TMR and TMH at different locations along the lower eyelid margin and analyses the influence of LIPCOF on tear meniscus regularity.

5. Finally, Chapter 8 evaluates the potential of the PDM to detect changes in lower tear meniscus after the application of artificial tears and analyses the influence of blinking on tear volume loss.

CHAPTER 4: Development of a new Portable Digital Meniscometer

4.1. Introduction

The aim of this PhD project was to improve the evaluation of the tear meniscus for the clinician. The first step to realise this aim was the development of a new simple instrument, suitable for use by any clinician.

Reflective meniscometry is a non-invasive, precise and repeatable measurement of the tear meniscus radius[26, 27, 35-37, 234]. As tear meniscus radius is correlated with tear volume, reflective meniscometry is an effective tool in the diagnosis of aqueous deficient dry eye[28, 29]. Nevertheless, worldwide, only three video-meniscometers are in use and at present the instrument is not commercially available. Although meniscometry has all the given advantages over other methods of tear meniscus evaluation, it is not widely used. This might be explained by the fact that for the measurement of tear meniscus radius with a video-meniscometer, a separate, specialised, and relatively unwieldy, device is necessary. Therefore, in 2007 the Diagnostic Methodology Subcommittee of the International Dry Eye Workshop described the adaptation of the meniscometry for general use[270], with the intent of developing a portable and affordable meniscometry device, which could be easily integrated in a routine eye examination at the slit-lamp.

4.2. The Portable Digital Meniscometer (PDM)

4.2.1. Projection Target

To project a target onto the anterior curvature of the tear meniscus, an illuminated target was needed. A conventional iPod-touch (Apple Inc., Cupertino, CA, USA) with a 3.5” multi-touch-display 7.5 x 5.0 cm (480 x 320 Pixel) was used for this purpose. An application software for the iPod-touch was developed to generate a grating of parallel black and white bands on the display (Figure 4.1). The width of the lines is shown on the display and can be varied between 0.15 and 15.0 mm via the touch screen (Figure 4.2). Preliminary work indicated that the optimal spacing of the grating was 7.5 mm for visibility and contrast. Additionally, the vertical orientation of the iPod is given in degrees at the display. This allows adjusting the target in different orientations to the tear meniscus.

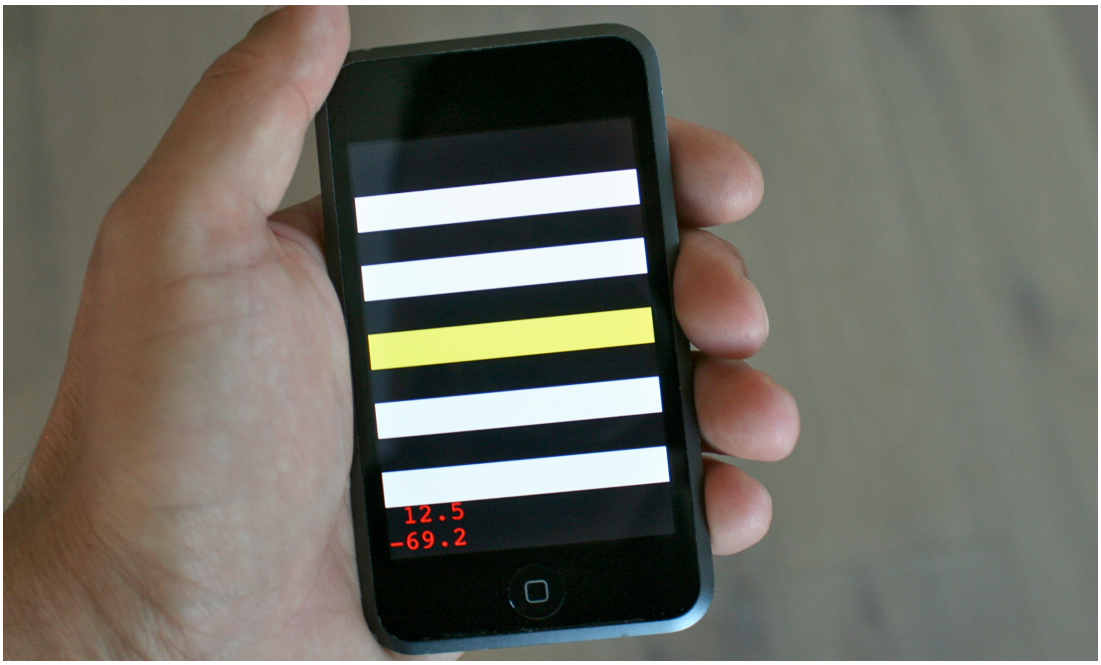


Figure 4.1: iPod-touch as a target with adjustable grid wide. Red numbers on the touch screen give the width of the bars in mm and the vertical orientation of the instrument in degrees.

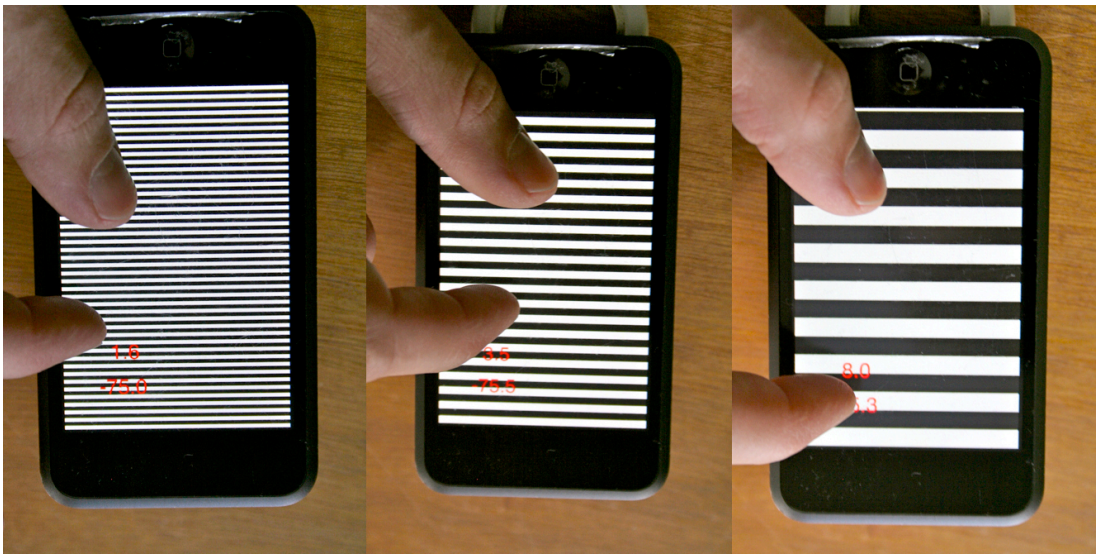


Figure 4.2: iPod-touch (Apple Inc., Cupertino, CA, USA) as a target with adjustable grid width.

4.2.2. Slit-Lamp Holder

To define the distance from the tear meniscus, the iPod-touch was fixed to a photo slit-lamp. A commercially available iPod-Touch stand (Xtand, Just Mobile e.K., Berlin, Germany) was modified and mounted on a metal axis on the stand so that it could be fixed to the tonometer post of the slit-lamp (Figure 4.3). This set-up allowed adjustment of the target in several orientations in relation to the tear meniscus. The target was presented to the tear meniscus with the grating bands disposed horizontally in the following studies.

Figure 4.3: Instrument mounted on the digital Haag-Streit BQ900 (left side), Topcon SL-D7 (middle), and a Zeiss SL-120 slit-lamp with a CCD-Camera (right side).

4.2.3. Distance and Angle of the Target

Specular reflection with the slit-lamp was achieved by setting the incidence angle of the target grating equal to the observation angle of the microscope (Figure 4.4), which was set at 40x magnification. Because the specular reflex is never observed simultaneously through both eyepieces, the eyepiece that belongs to the camera image needs to be chosen. The angle between target and observation system was controlled with a protractor. According to the literature, an angle of around 20° between the target and sagittal plane, and between the observation system and sagittal plane was chosen. The distance between the target (iPod) and the glass-capillaries (a = target distance) was controlled using a sliding calliper. Once the image of the reflective grid was sharp on the computer display, the distance between

the target and the glass-capillaries was measured. The optimal working distance (a) was found to be 50 mm.

Figure 4.4: Patient positioned in front of the portable, slit-lamp mounted, digital meniscometer (PDM). The grid on the screen of the iPod touch is reflected by the cornea and the lower tear meniscus.

4.2.4. Imaging and Radius Calculation

Imaging of the reflection was achieved using a digital camera (e.g. RM 01 CCD-camera, 1600 x 1200 pixel, Haag-Streit, Koeniz, Switzerland) incorporated into the slit-lamp, and relayed to image-grabbing software (EyeSuite Imaging, Haag-Streit, Koeniz, Switzerland) within a PC. The computer screen had a resolution of 1280 x 1024, producing a total magnification of about 100x, which was the best compromise

in terms of resolution and brightness of the image. The images were saved as JPEGs and at a later point in time they were opened by ImageJ 1.46 software (http://rsbweb.nih.gov/ij) for analyses. On the image of the reflected grating obtained, the distance between the outer edges of two black lines (total width of two black and one white projected line) was measured using ImageJ (Figure 4.5). The central three lines were selected to minimise any impact of an eventually non-circular profile of the meniscus. With a known size of the target (y), distance of the target (a) and the size of the image on the screen (y'), the radius of the tear meniscus was calculated using the given formula for a concave mirror (Figure 4.6)[27]. For calculating the radius more easily, a calculation tool was written for Microsoft Excel (Figure 4.7).

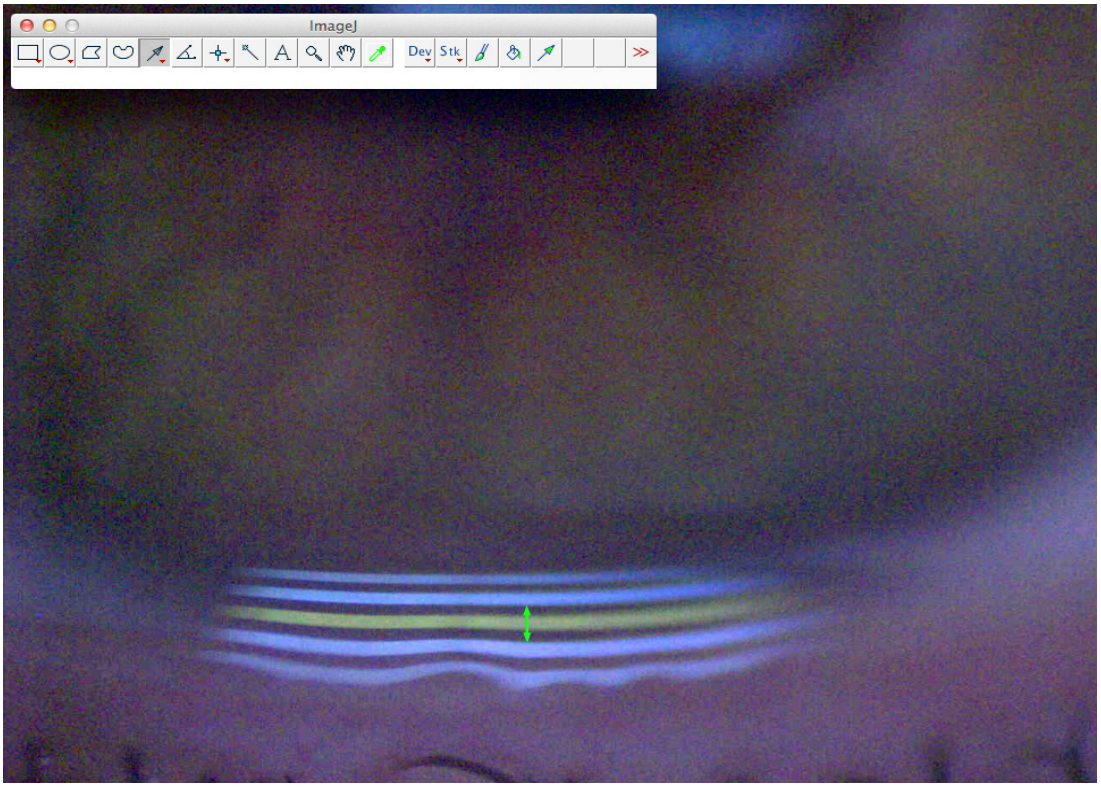
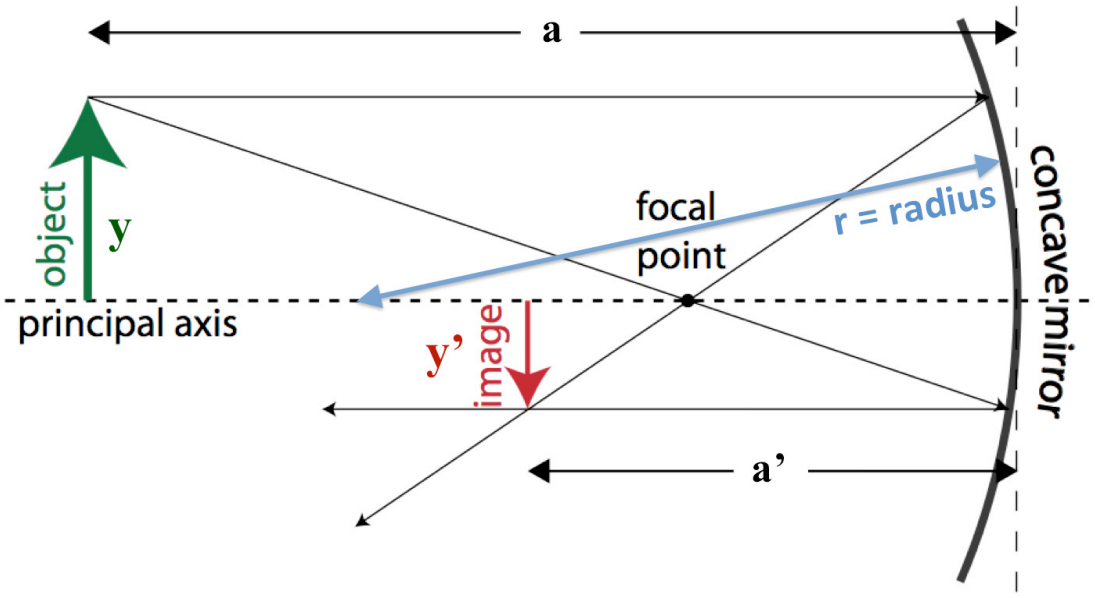


Figure 4.5: Measurement of line distance on the PDM-image using ImageJ 1.46 software.



$$r = \frac{2 \cdot a \cdot y'}{y - y'}$$

r = radius of meniscus curvature

a = target distance

y = target size

y' = image size

Figure 4.6: Concave mirror formula for calculation of the tear meniscus radius in reflective meniscometry.

Tear Meniscus Radius Calculator			
Target Distance	a =	55	mm
Target Size	y =	23	mm
Image Size	y' =	0,102	mm
Radius	r =	0,490	mm

Figure 4.7: Excel tool for calculation of tear meniscus radius

4.3. Discussion

The newly developed device uses the principal of the reflective video-meniscometer, but can be used on every commercially available digital slit-lamp. This follows the published recommendations of the Dry Eye Workshop that suggests the adaption of reflective meniscometry for general use[270]. A simple iPod touch or an iPhone can be used to project the necessary grid and only an additional holder is necessary to mount the system to any slit-lamp. So besides being mobile, the new system is extremely low-priced.

Furthermore, the system is used in combination with the slit-lamp, which means that several observations can be made at the same time. Thus the observation of tear meniscus height, with or without fluorescein, can be made simultaneously with the

measurement of tear meniscus curvature at the same location along the lid (Figure 4.8).

Compared to the classic meniscometer, where only a few lines can be observed on the meniscus, the new instrument allows the reflection of up to 12 lines at the meniscus, which means that irregularities in the shape of meniscus are made visible (Figure 4.9). Another great advantage is the capability of the system to generate videos of the tear meniscus. This means that real time changes of the meniscus following a blink and in-between blinks can be made visible.

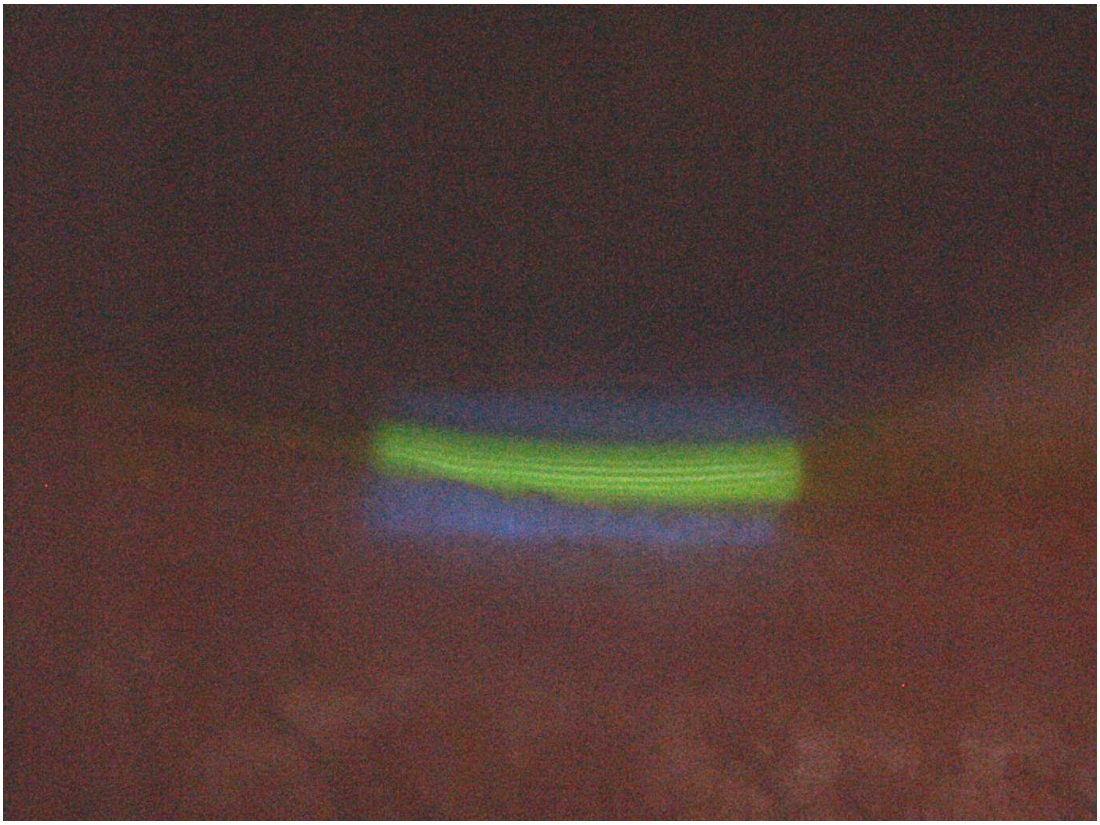


Figure 4.8: Simultaneous observation of tear meniscus height (with fluorescein) and tear meniscus radius.

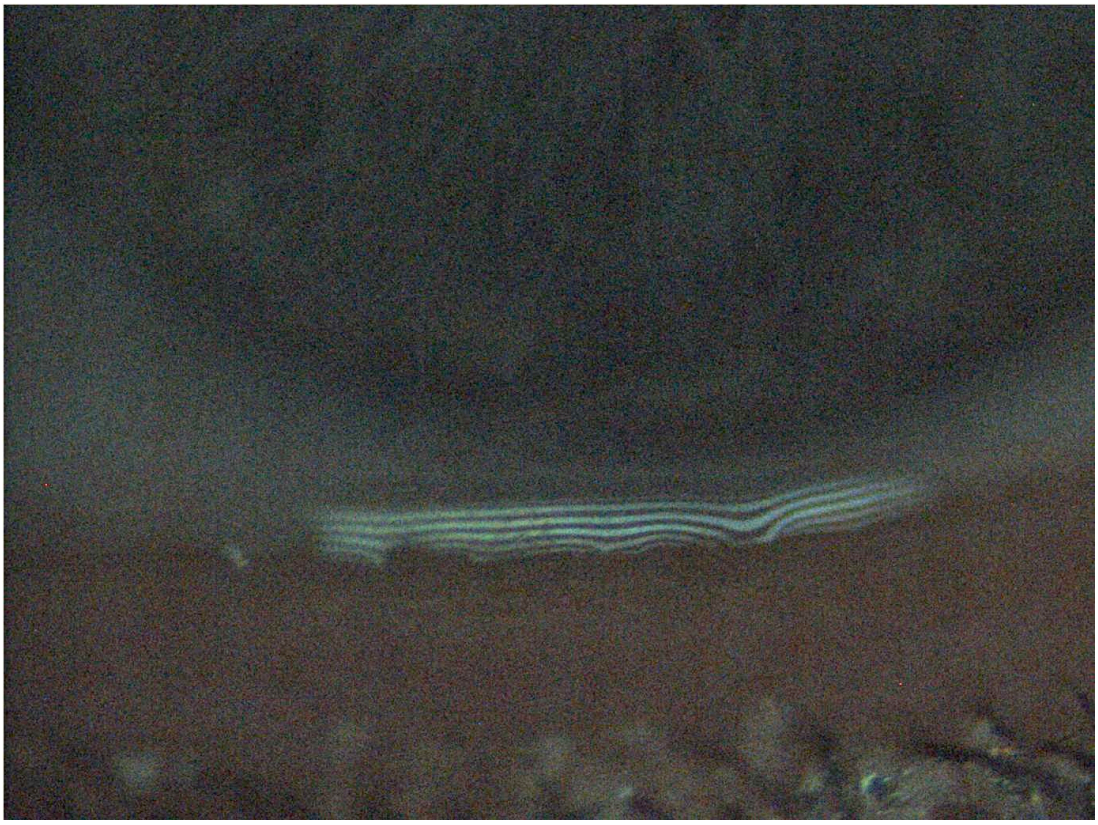


Figure 4.9: Irregularities in meniscus reflex and shape.

4.4. Conclusions

A portable, slit-lamp mounted, digital meniscometer based on an iPod touch has been developed. This new instrument is a simple, mobile and reasonable device to measure tear meniscus radius, and therefore tear volume, and is suitable for use by clinicians.

CHAPTER 5: Accuracy and Repeatability of the new Portable Digital Meniscometer

5.1. Introduction

This study aims to evaluate the newly developed portable digital meniscometer using *in-vitro* and *in-vivo* experiments. The results of this study will provide the clinician with information on the accuracy and repeatability of this new device. As a reference instrument in this study, the Yokoi et al.[29] video-meniscometer was used.

Dry eye is a multi-factorial disease resulting in damage to the ocular surface and symptoms of discomfort, principally due to an aqueous deficiency or to increased tear evaporation[347]. The superior and inferior tear menisci together represent 75 to 90% of the total tear film volume[183], although a lower estimate of 27% has also been made[348]. It has been shown that the lower tear meniscus curvature is directly related to tear volume[6], which, in turn, is related to tear flow rate[62]. Thus, various tear meniscus parameters, such as radius of curvature and height, which are indicators of the tear film volume, are important in the diagnosis of aqueous-deficient dry eye[29, 31, 34, 189, 349]. Measurement of tear meniscus height has been used in many studies as a surrogate for tear volume and, in clinical practice, is mostly performed with a slit-lamp[19-23, 200]. However, identifying the upper limit of the meniscus at the slit lamp is challenging unless sodium fluorescein is added to the tear film, which in turn renders the test invasive and may introduce errors.

In contrast, the radius of tear meniscus curvature (TMR), while more difficult to measure, may be better at predicting tear volume, since it is performed in a non-invasive manner[26-29, 234]. TMR can be evaluated by the use of slit-lamp photography[189], optical coherence tomography (OCT)[28, 30-33, 240], or meniscometry[26, 27, 34-37, 214]. Although both OCT and meniscometry measure the tear meniscus radius non-invasively, they have not found wide application amongst clinicians, either because they are not commercially available in all parts of the world, or they are too expensive[14].

As described in Chapter 2, the first photographic meniscometer was introduced by Bron[235] in 1997, and reported by Yokoi et al.[27] in 1999. A modification of the video system, called the "video-meniscometer", was developed by Yokoi et al.[34, 37], which used a target consisting of a series of black metal bars, 4 mm wide and 4 mm apart, set directly in front of the objective lens and illuminated from behind. However, only three versions of the free-standing video-meniscometer that were developed from this prototype by Oguz et al.[37] were produced and remain in use.

Another attempt with a prototype of a meniscometer, named a "dacryomeniscometer", was introduced by Ho et al.[214]. While this instrument was originally designed to describe the tear meniscus profile, it was used only for tear meniscus height measurements in later studies[143, 188].

The aim of this study was to test the accuracy and repeatability of the newly developed portable digital meniscometer compared to the Yokoi et al.[29] video-meniscometer.

5.2. Methods

5.2.1. *In vitro* study

The inner surfaces of 5 glass capillaries were used as a model of the tear meniscus. The inner diameters and the circularity of the inner surface of the glass capillaries (Hilgenberg GmbH, Malsfeld, Germany) (Figure 5.1) were confirmed by use of a hole-gauge (W&Z-Computer-Vertrieb GmbH, Dresden, Germany) (Figure 5.2) before cutting them length-wise in half. Based on preliminary studies, the medians of three consecutive measurements on the 5 glass capillaries (radii 0.100 mm to 0.505 mm) were compared between the existing video-meniscometer (VM) (Figure 5.3) and the new portable digital meniscometer (PDM), as described in Chapter 4.2. The PDM was fixed on a BQ900 slit-lamp with IM900 digital imaging module (Haag-Streit, Koeniz, Switzerland). The measurements were compared at two different sessions at the same time of day (day 1 and day 2), and after re-set-up of the PDM.

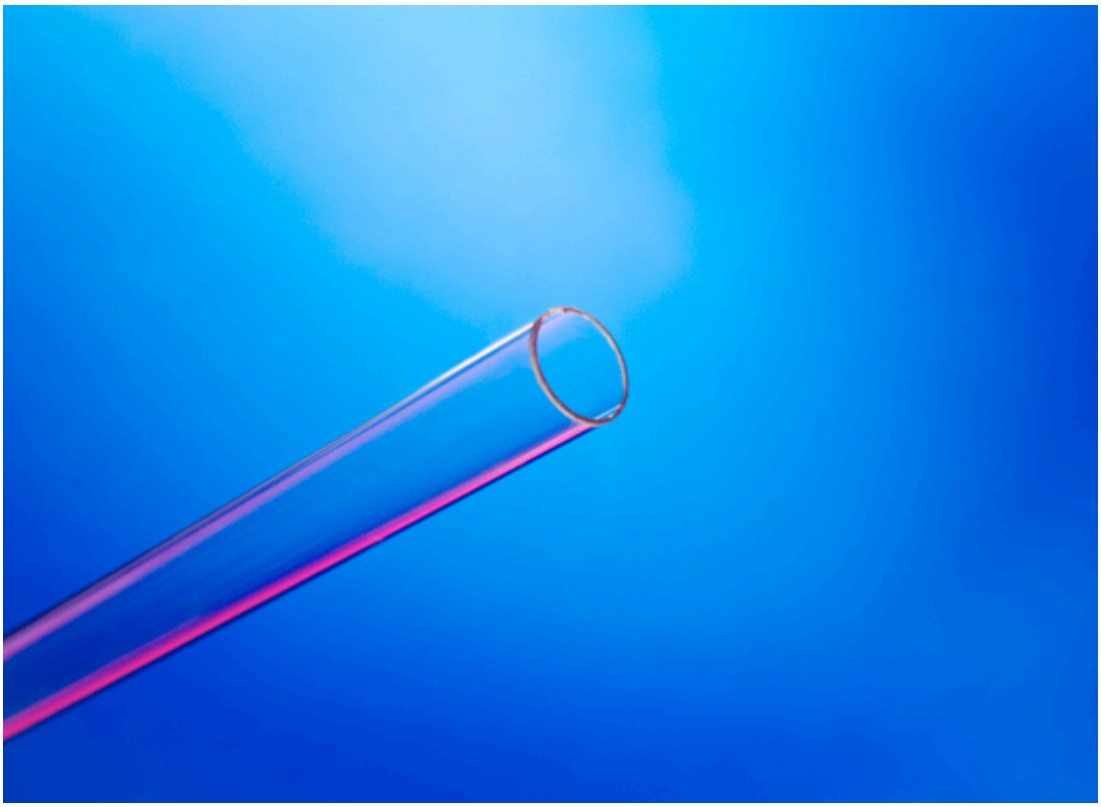


Figure 5.1: Glass capillaries (Hilgenberg GmbH, Malsfeld, Germany) before cutting them length-wise in half. The inner surface was used as a model of the tear meniscus anterior surface.

Figure 5.2: Hole gauge with a conical head to measure the inner diameter of the glass capillaries.

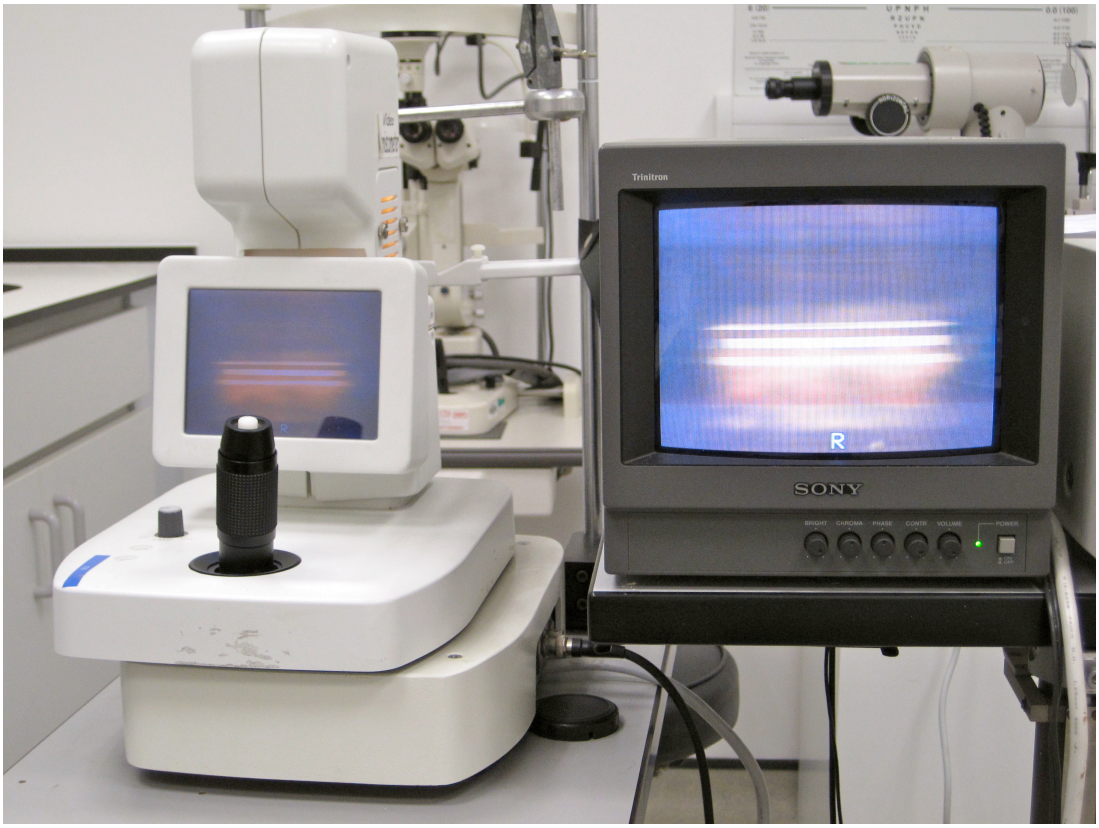


Figure 5.3: Yokoi et al video-meniscometer.

5.2.2. *In vivo* study

Twenty subjects (male = 10, female = 10, mean age 32.3 years, range = 23-56 years) were randomly selected from the students and staff of the School of Optometry and Vision Sciences at Cardiff University, UK. All procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Research Audit Ethics Committee and were conducted in accordance with the requirements of the Declaration of Helsinki. All subjects gave written informed consent before participating in the study.

Subjects were excluded if they were pregnant or breastfeeding; had a current or previous condition known to affect the ocular surface or tear film; had a history of previous ocular surgery, including refractive surgery, eyelid tattooing, eyelid surgery, or corneal surgery; had any previous ocular trauma, were diabetic, were taking medication known to affect the ocular surface and/or tear film, and/or had worn contact lenses less than two weeks prior to the study. Subjects with a history of dry eye, defined by either an item-weighted McMonnies questionnaire score >14.5 or a fluorescein tear break-up time <10 seconds, were excluded[324, 350].

The lower TMR was measured by one observer using both techniques (VM and PDM) in a randomised order. Care was taken to align both instruments consistently for each data collection. Based on pre-experiments, the median of three consecutive measurements was recorded for both techniques, instead of the mean. For both techniques the measurement time was about two minutes with a break of one minute between the two instruments. All assessments were of the inferior tear meniscus of the right eye, directly below the pupil centre, with the subject looking straight ahead at a fixation target. The room temperature range was 18 to 22°C and the relative humidity 30-40%. To minimise the effect of diurnal and inter-blink variation, measurements were taken in the morning between 10 and 12 o'clock and 3 to 4 seconds after a blink.

5.3. Statistical analyses

Normal distribution of data was analysed by the Shapiro-Wilk test. Differences between sessions (day 1 and day 2) and instruments were analysed using Bland-Altman plots, coefficient of repeatability (CR), and paired t-tests. The relationship between PDM and VM measurements was analysed by Pearson product moment correlation. The data were analysed using SigmaPlot 12 (Systat Software Inc., Chicago, USA) and BiAS 10 (epsilon-Verlag, Darmstadt, Germany).

5.4. Results

5.4.1. *In vitro* study

The median measured radii of the 5 glass capillaries were 0.105, 0.186, 0.349, 0.394, 0.503 mm for the PDM, and 0.088, 0.169, 0.342, 0.403, 0.534 mm for the VM. The mean difference between the measurements of the two devices was 0.0002 mm (95% CI –0.0252 to +0.0256 mm; p=0.984) (Figure 5.4).

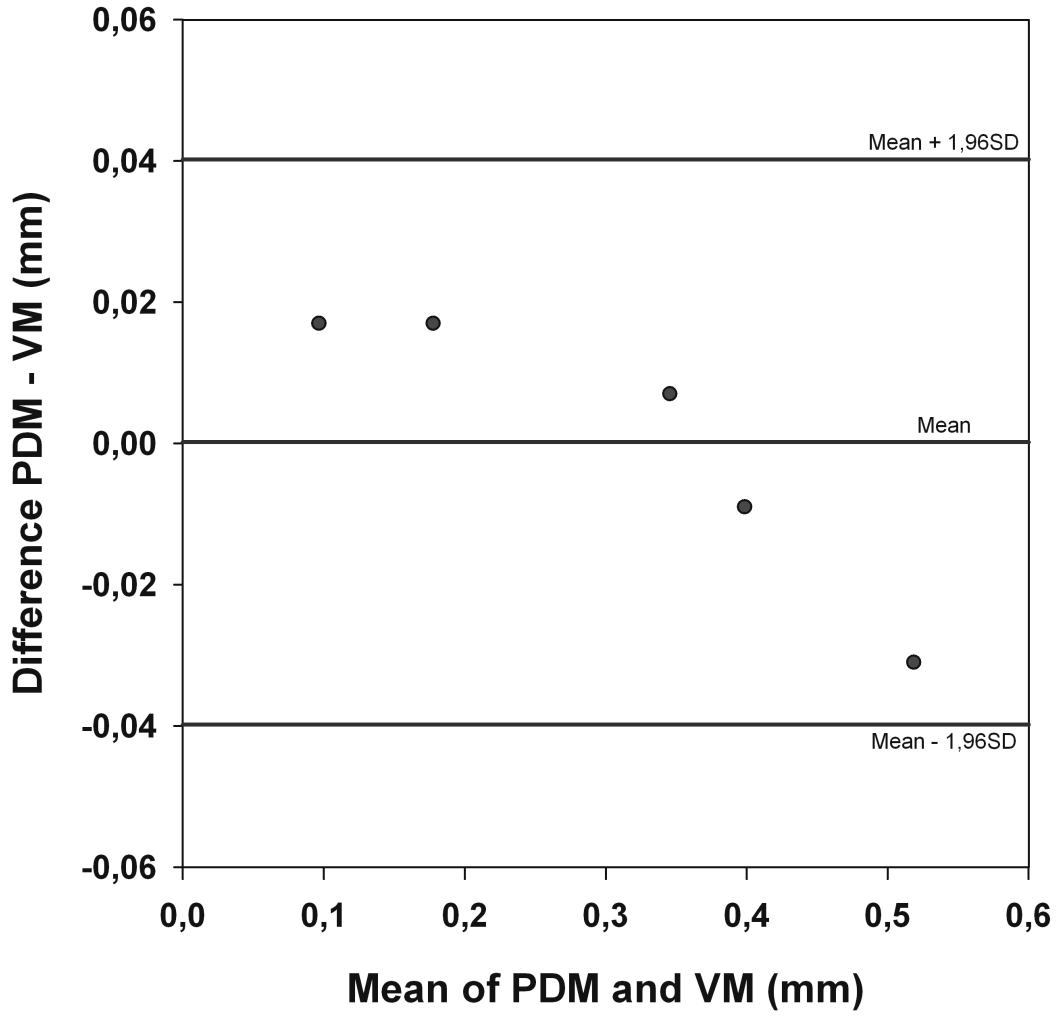


Figure 5.4: *In vitro* radii differences between PDM and VM.

Repeated measurements between day 1 and day 2 were not significantly different for the PDM and VM (paired t-test; p=0.468 and p=0.775, respectively). The 95% confidence intervals around differences indicate acceptable repeatability (95% CI: PDM -0.0134 to +0.0074 mm; VM -0.0282 to +0.0226 mm), and reproducibility between sessions (95% CR: 0.019 mm and 0.018 mm, PDM and VM respectively) (Figures 5.5 and 5.6).

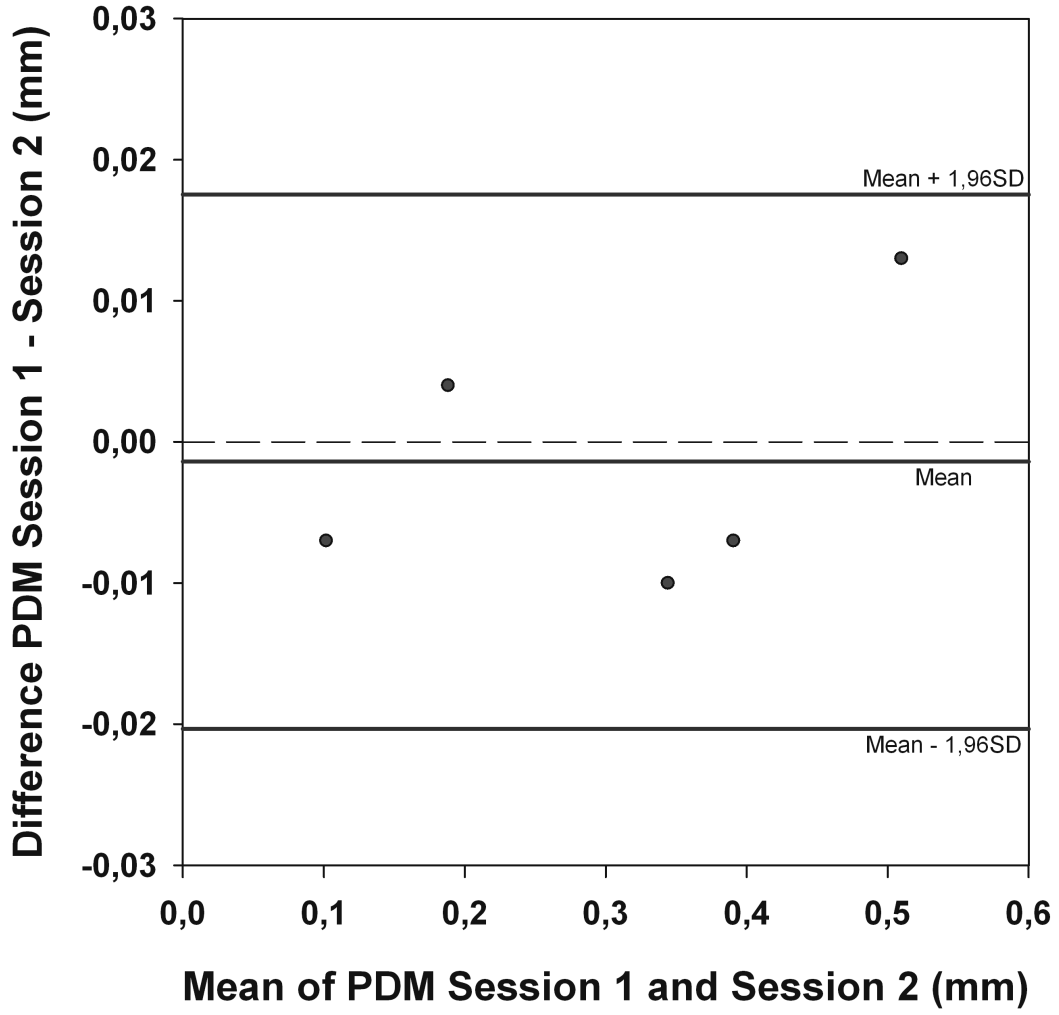


Figure 5.5: *In vitro* radii differences between sessions for the PDM.

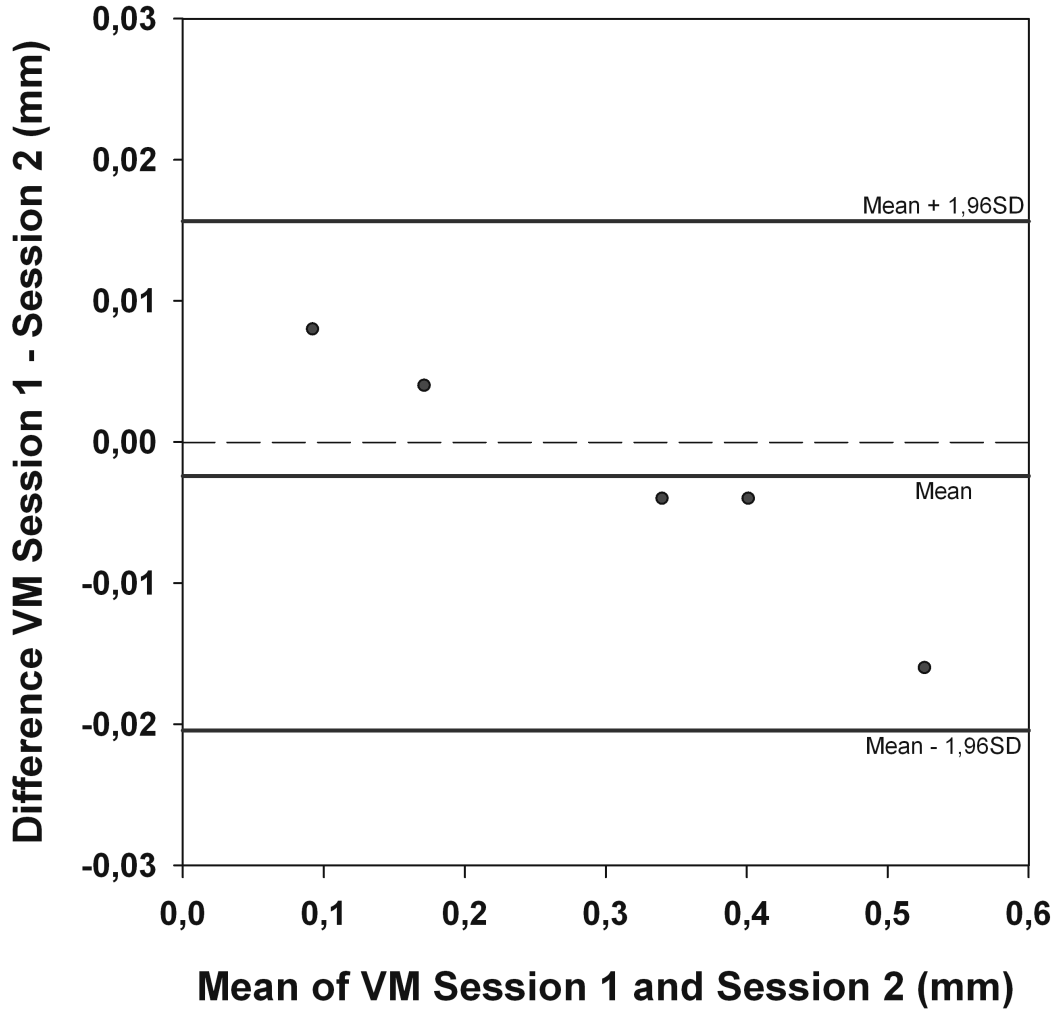


Figure 5.6: *In vitro* radii differences between sessions for the VM.

Examples of the PDM target reflection from a steep (r=0.349 mm) and a flat capillary inner radius (r=0.503 mm) are shown in Figures 5.7a and 5.7b.

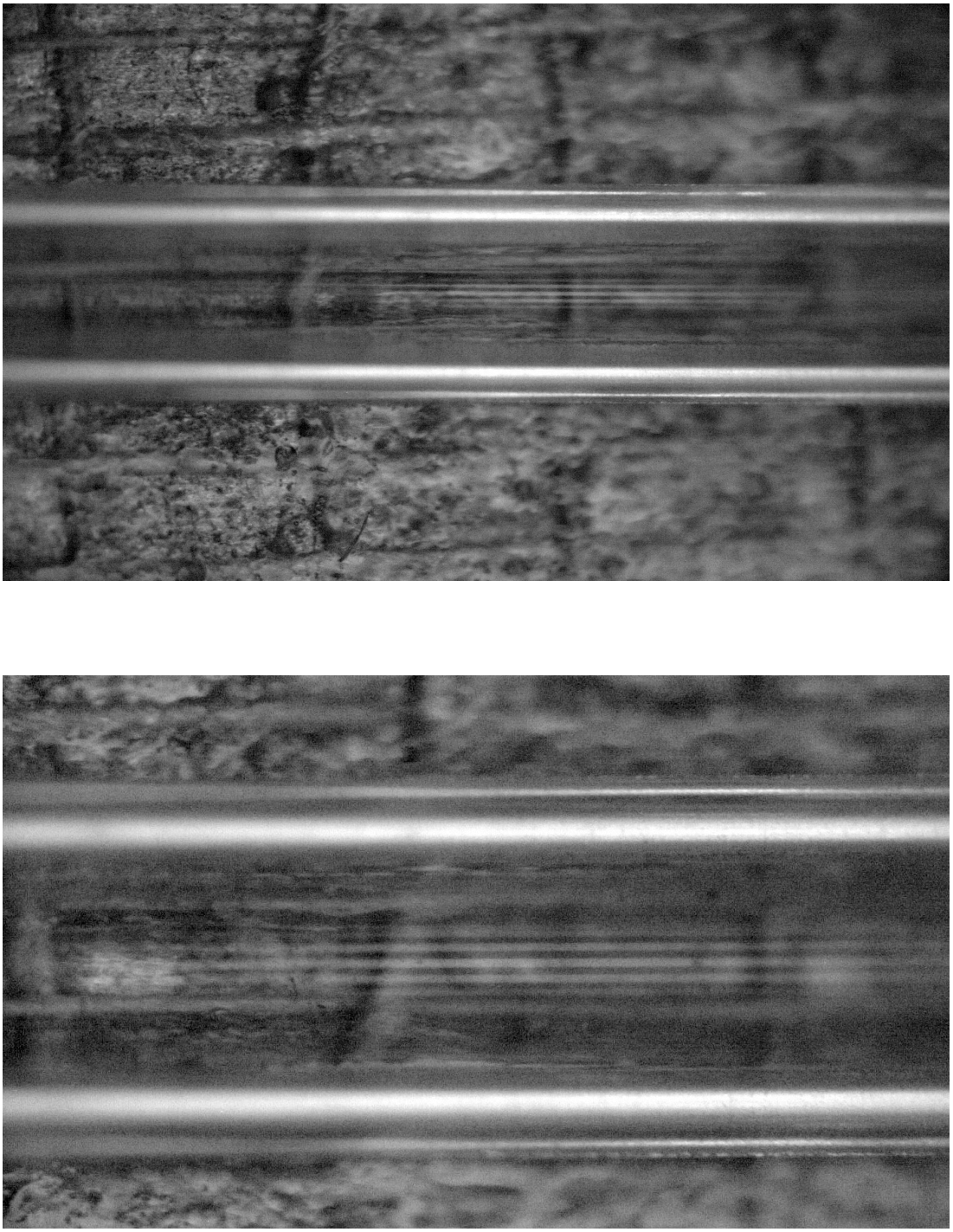


Figure 5.7: Example of PDM target reflection from (a) a steep (r=0.349 mm) and (b) a flat capillary inner radius (r=0.505 mm).

5.4.2. *In vivo* study

The mean TMR of the subjects measured with the PDM was 0.34±0.10 mm and 0.36±0.11 mm of the VM. PDM measurements were significantly correlated to measures of the VM (Pearson product moment correlation; r=0.940; p< 0.001). There was a non-significant difference between the measurements taken by the PDM and the VM (mean difference -0.0151 mm; 95% CI: -0.0285 to -0.0018 mm; paired t-test; p=0.124) in this cohort (Figure 5.8). The power calculation of the completed *in vivo* study resulted in a power of 0.97 (α=0.05).

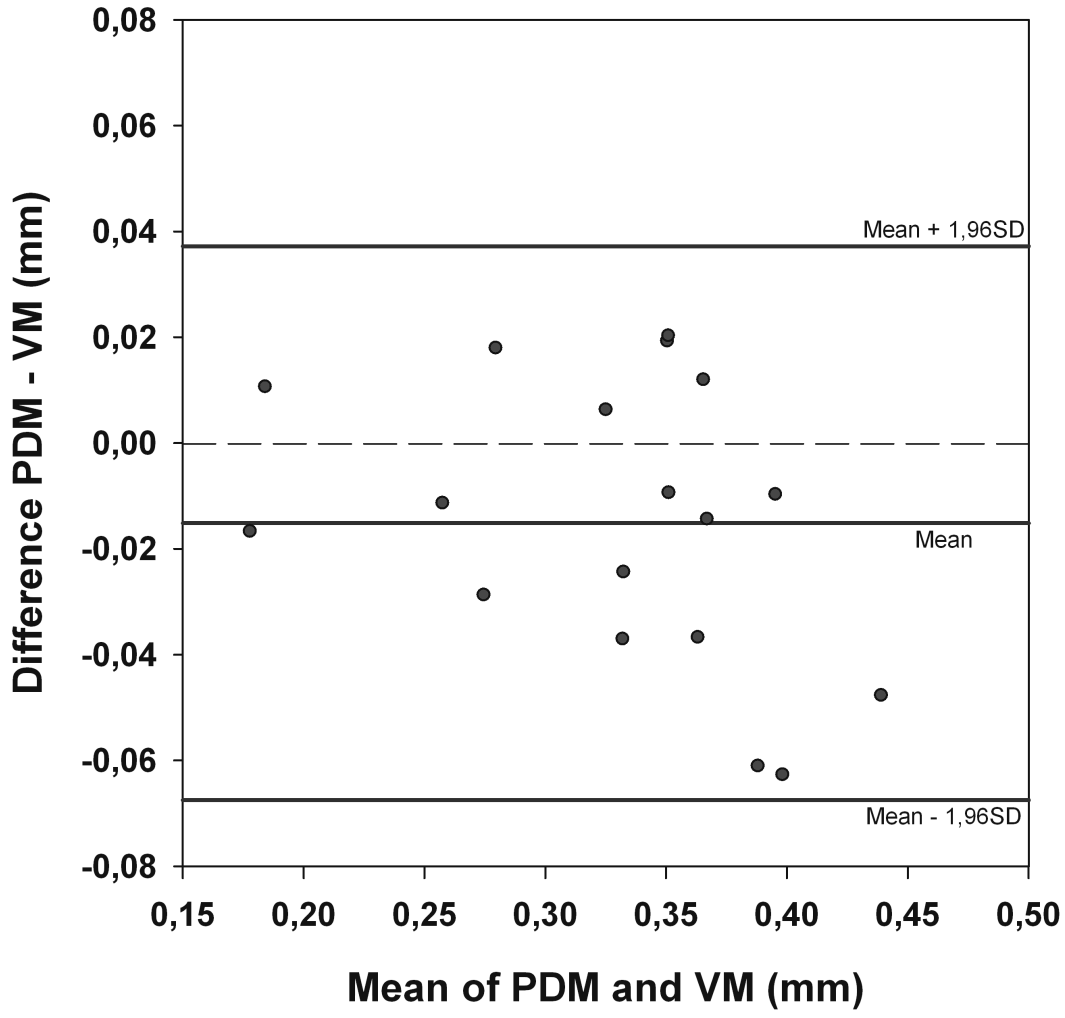


Figure 5.8: *In vivo* radii differences between PDM and VM.

Examples of the PDM target reflection from a steep (r=0.19 mm) and a flat tear meniscus radius (r=0.37 mm) are shown in Figures 5.9a and 5.9b.

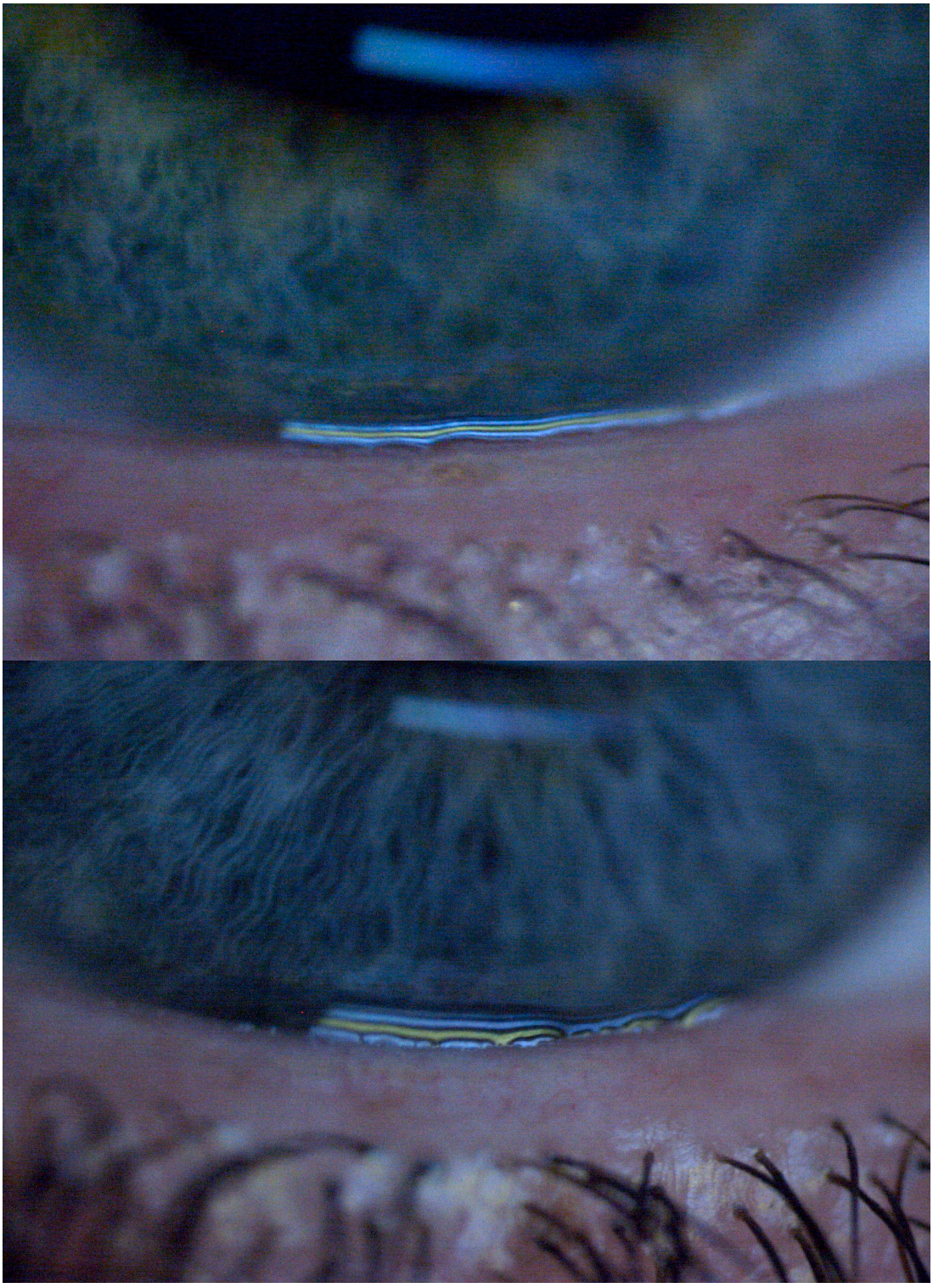


Figure 5.9: Example of PDM target reflection from (a) a steep tear meniscus radius (r=0.19 mm) and (b) a flat tear meniscus radius (r=0.37 mm).

5.5. Discussion

With the newly developed iPod-touch based, portable, slit-lamp mounted meniscometer a good accuracy and reproducibility across the whole range of typical TMR values was found (Figure 5.5). In contrast, the VM had a tendency to under-estimate the TMR for small radii and to over-estimate TMR for larger radii (Figure 5.6).

This pattern of results was also evident in the comparison between the two methods when the radii measured, with the PDM being more consistent than the VM (Figure 5.4). Since the experimenter was trained in maintaining the alignment of both devices, these apparent differences might be caused by differences in the design and presentation of the targets. While the VM uses metal bars, mounted coaxial with the observation system, the target of the PDM consists of digitally generated bands, which are separated from the observation system. As a result, the PDM target does not interfere with the observation system of the slit-lamp, since the VM target effectively functions as an aperture within the observation system, thus influencing the depth of field. A second source of error arises from the working distance of the instrument. While the VM has a working distance of 24 mm, a longer distance of 50 mm is used by the PDM. By looking at the concave mirror formula it becomes obvious that the smaller the working distance, the greater the error, if the system is not exactly aligned.

In vivo, there was a good agreement between the TMR values of the two instruments. With the PDM, a TMR of 0.34 ± 0.10 mm was found in this group of healthy, non-dry eye patients. This was not significantly different from the TMR measured with

the VM (0.36 ±0.11 mm) and is in accordance with previously reported measurements using reflective meniscometry in non-dry eye subjects[27, 34]. The correlation between the two methods indicates that the PDM provides a valid measurement of TMR. For dry eye patients the reported TMR, measured by reflective meniscometry, has varied between 0.22±0.09 and 0.25±0.09 mm[29, 34, 37], although some of these reports related to patients with evaporative dry eye.

While meniscometry uses specular reflection to analyse TMR, in optical coherence tomography a vertical line scan produces a cross-sectional image of the tear meniscus. On the images taken with an OCT, the 3-point method is used to fit a circle to the anterior border of tear meniscus. TMR of the lower tear meniscus reported with this method varies from 0.25±0.05 to 0.46±0.40 mm for normals and between 0.15±0.03 to 0.20±0.08 mm in dry eye patients[28, 30, 32, 164, 240]. As in this study, calibration of the original meniscometer system was carried out using glass capillaries[27]. Also using glass capillaries, Kato et al.[351] found no significant differences between TMR measured with the VM and an anterior segment optical coherence tomographer.

For the purpose of calculating meniscus volume, the anterior shape of the meniscus is treated as a part of a circle even though it is likely to have a more complex shape[184]. To understand differences in TMR measurements between reflective meniscometry and optical coherence tomography it would be helpful to describe the shape of the meniscus more precisely and to analyse the location on the meniscus were the PDM is measuring the meniscus. While commercial OCT and the existing VM have a fixed orthogonal orientation of the target, the PDM allows rotation of the

target and therefore a measurement of the meniscus under different angles in the coronal plane. This could be of value in following differences in TMR along the nasal and temporal slopes of the lid. Furthermore, the band-width of the target can be easily varied via the touch screen. This enables a finer grating to be projected onto the meniscus, with the possibility of obtaining a more detailed description of the tear meniscus profile.

5.6. Conclusions

Measuring TMR is a useful non-invasive test for dry eye diagnosis[27, 28, 34, 234, 240], but existing techniques are either not available commercially or are too expensive for general clinical use. The portable, slit-lamp mounted, digital meniscometer permits accurate and reliable measurements of human tear meniscus radius. The PDM can be made generally available and is suitable for use in both research and clinical practice.

The published form of chapters 4 and 5 can be found in Appendix 2.1:

Bandlitz S, Purslow C, Murphy PJ, Pult H, Bron AJ.

A new portable digital meniscometer.

Optom Vis Sci 2014;91:e1-8.

CHAPTER 6: Comparison of a new portable digital meniscometer and optical coherence tomography in tear meniscus radius measurement

6.1. Introduction

Beside reflective meniscometry, optical coherence tomography allows a non-invasive evaluation of the tear meniscus radius. Therefore, this study will compare these two techniques and help to answer the questions: (i) is there an agreement between the new PDM and OCT in the measurement of the TMR; and (ii) where is the location on the tear meniscus from which the PDM image is being reflected? The results of this study will be useful for a better understanding of the measuring principles of OCT and the PDM, and hence maintain the analysis of the results in the later experiments.

The tear fluid on the ocular surface is present in the exposed area between the lids, in the conjunctival sac of the upper and lower lids, and in the tear menisci along the lid margins. However, the tear menisci hold approximately 75% to 90% of the overall tear fluid volume and serve as reservoirs, supplying tears to the pre-corneal tear film[32, 183, 348]. The measurement of the anterior curvature radius of the tear meniscus (TMR) is an indicator of tear film volume and has been found to have good dry eye diagnostic accuracies[27, 28, 34, 37, 189, 234]. When TMR measurement is done in a non-invasive way, this method has great advantages over other invasive tests to evaluate aqueous tear production or volume. These invasive tests, like the Schirmer and

Phenol red thread tests, are variably influenced by reflex tearing and show large variations in the test results[194, 259].

TMR can be measured using a slit-lamp microscope image capture system[163, 189, 203], optical coherence tomography (OCT)[28, 30-33, 240], or reflective meniscometry[26, 27, 34-37]. With the slit-lamp bio-microscope, the radius of the meniscus can be observed in cross-section. TMR is normally assessed on the captured image by determining the radius of a circle that best fits the curved anterior meniscal face, with sodium fluorescein instilled in the tear film to improve visibility of the anterior border of the meniscus, although the addition of fluorescein dye will increase tear volume and influence tear meniscus radius[163, 189, 203, 232]. Indeed, the values of TMR obtained from this image capture technique with fluorescein are typically larger than those reported with reflective meniscometry or OCT (Table 6.1).

Reflective Meniscometry			**Optical Coherence Tomography**			**Slit Lamp**		
Author	**Lower TMR**	**Subjects**	**Author**	**Lower TMR**	**Subjects**	**Author**	**Lower TMR**	**Subjects**
Yokoi et al. (1999)	0.37 ± 0.15	45.6 ± 21 years (n=45)	Wang et al. (2008b)	0.46 ± 0.40	45.1 ± 15.4 years (n=36)	Johnson and Murphy (2006)	0.48	29 (20-53 years) (n=15)
Maruyama et al. (2004)	0.26 ± 0.09	23.5 ± 5.2 years (n=11)	Palakuru et al. (2007)	0.39 ± 0.31	32.1 ± 8.7 years (n=21)	Golding et al. (1997)	0.48 ± 0.21	
Bandlitz et al. (2010)	0.34 ± 0.10	32.3 ± 9.3 years (n=20)	Wang et al. (2006)	0.26 ± 0.15	40.5 ± 14.1 years (n=20)	Mainstone et al. (1996)	0.55 ± 0.26	64.4 ± 11.1 years (n=15)
			Wang et al. (2008a)	0.29 ± 0.16	31.7 ± 8.9 years (n=20)			
			Shen et al. (2009)	0.25 ± 0.05	38.5 ± 12.7 years (n=47)			
			Li et al. (2012)	0.24 ± 0.05	33.3 (19-56 years) (n=48)			
Mean ± SD [mm]	**0.32 ± 0.11**			**0.32 ± 0.19**			**0.50 ± 0.24**	

Table 6.1: Mean ± standard deviation of central lower tear meniscus radius (TMR) values [mm] of normal subjects reported in the literature using reflective meniscometry, optical coherence tomography and slit-lamp image capture technique.

In contrast, reflective meniscometry is a non-invasive technique that measures TMR, as described in Chapter 2.

Anterior segment OCT of the ocular surface also permits a non-invasive examination of the tear meniscus[33, 172, 204]. OCT provides cross-sectional high-resolution images of the meniscus and can be applied to the diagnosis and evaluation of dry eye disease[28, 137, 173, 205, 240, 342, 352, 353]. Although OCT is useful for tear meniscus measurements, it has not found wide application among clinicians, mainly because it is considered to be too expensive[14]. On an OCT image, TMR can be measured using the three-point method to fit a circle, thereby producing the tear meniscus radius[33], although there are some issues in determining the accuracy of these measurements, due to the assumptions made in the instrument image processing algorithms. As with the slit-lamp image capture system, the anterior profile of the meniscus on the cross-sectional OCT images is treated as part of a circle with just one radius from the top to the bottom. However, it is likely that the profile of the meniscus has a more complex shape[184]. Using slit-lamp image capture to analyse changes in TMR after a blink, Johnson and Murphy[163] sub-divided TMR into two radii, one at the top and one at the bottom of the meniscus. A more detailed description of the radii at the anterior surface of the meniscus has not been addressed in other OCT studies.

Based on the technique of reflective meniscometry for measuring TMR, a new portable, slit-lamp mounted, digital meniscometer (PDM) was developed as described in Chapter 4[354]. The PDM uses a novel method using an iPod or iPhone screen to produce an illuminated target of parallel black and white bands which is then projected onto the meniscus at the lower lid margin. The PDM technique has

been shown to be accurate and reliable, and is able to provide similar values for TMR to the existing non-portable video-meniscometer (VM)[354] (see Chapter 5). Since the costs for the PDM are relatively low in comparison to the VM and OCT, it is suggested for use in both research and clinical practice.

Whilst VM and PDM both use reflective meniscometry to measure TMR, OCT uses a different technique. A more detailed description of the shape of the meniscus using a cross-sectional OCT image might therefore help in our understanding of the reflection-based principle of the PDM, specifically the region of the tear meniscus that the PDM image is reflected from. So, the aims of this study were (i) to investigate the agreement between the new PDM and OCT in the measurement of the TMR, and (ii) to analyse the location on the tear meniscus from which the PDM image is being reflected.

6.2. Methods

6.2.1. Subjects

Thirty healthy subjects (male = 8, female = 22) were randomly selected from the staff and students of the Höhere Fachschule für Augenoptik Köln, (Cologne School of Optometry), Cologne, Germany. The mean age was 27.5 years (standard deviation, ±9.3 years; range, 20 to 65 years). Subjects were excluded if they were pregnant or breast-feeding; had a current or previous condition known to affect the ocular surface or tear film; had a history of previous ocular surgery, including refractive surgery, eyelid tattooing, eyelid surgery, or corneal surgery; had any

previous ocular trauma, were diabetic, were taking medication known to affect the ocular surface and/or tear film, and/or had worn any types of contact lenses less than two weeks prior to the study. Subjects with a history of dry eye, defined by either an item-weighted McMonnies questionnaire score >14.5 or a fluorescein tear break-up time <10 seconds, were excluded[324, 350]. All subjects gave written informed consent before participating in the study. All procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Research Audit Ethics Committee and were conducted in accordance with the requirements of the Declaration of Helsinki.

6.2.2. Instruments

The portable digital meniscometer (PDM), as described in Chapter 4.2., was used to measure the central inferior tear meniscus radius. The PDM was mounted on a digital imaging slit-lamp (BQ900 with IM900 digital imaging module, Haag-Streit, Koeniz, Switzerland).

The OCT images were obtained using a Cirrus HD-OCT (Carl Zeiss Meditec, Jena, Germany). This instrument uses spectral domain OCT (SD-OCT), with a wavelength of 840nm to achieve an axial resolution of 5μm. The cross-sectional images of the tear meniscus in this study were taken using the anterior segment five lines raster method (Figure 6.1). In this mode, five parallel vertical lines of 3 mm length and a line distance of 0.25 mm were scanned; each line was composed of 4096 A-scans. Since the anterior segment ruler function of the Cirrus HD-OCT is calibrated to measure in a vertical direction within corneal tissue, the images were rotated 90° before measuring the tear meniscus radius.

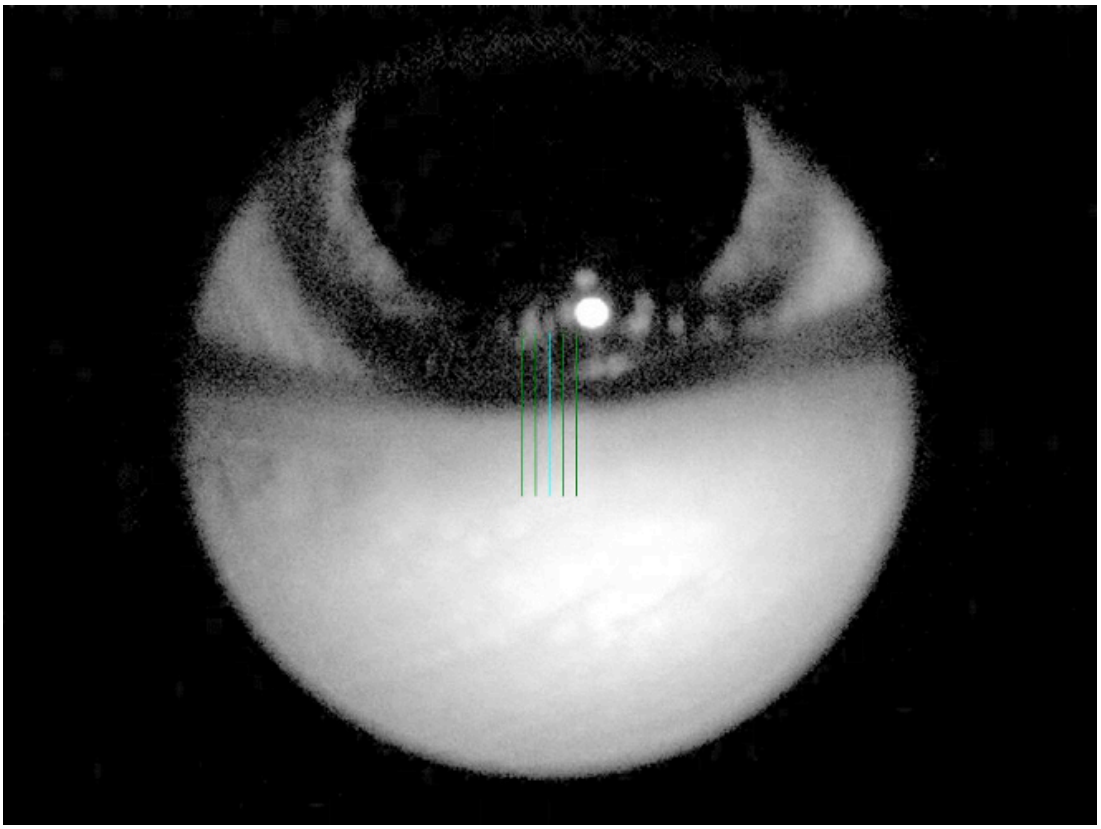


Figure 6.1: Tear meniscus cross sectional imaging with the anterior segment 5 line raster of the Cirrus HD-OCT, showing the observer's view and the alignment targets.

6.2.3. Sample calibration

To ensure that on-screen images represented curvature of known dimensions, the inner surface of five glass capillary tubes (radii 0.100 mm to 0.505 mm; Hilgenberg GmbH, Malsfeld, Germany) were used as a model for the tear meniscus. The inner diameters of the glass capillaries were confirmed by use of a hole-gauge before cutting them in half. Three OCT scans were taken for each glass capillary. The OCT images were then exported to ImageJ software. Within the ImageJ software, a circle of the confirmed radius of the capillaries was used as a template onto which the OCT image was stretched or compressed until it matched with the circle (Figure 6.2). A

regression line was then calculated to form a calibration curve, from which all OCT images of the tear meniscus were adjusted before analysis.

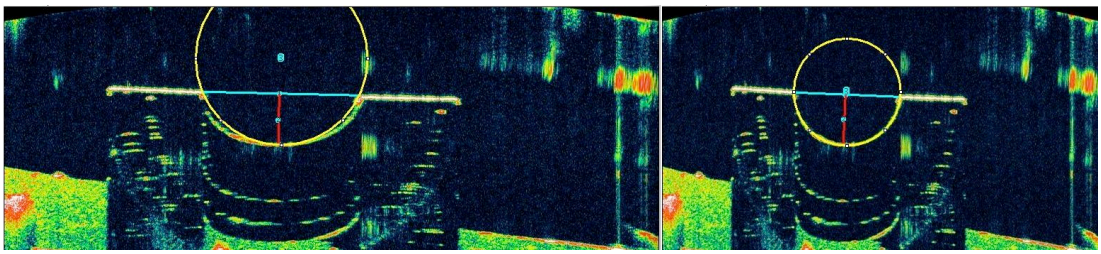


Figure 6.2: OCT image of a glass capillary before (left) and after (right) image adjustment. The red line represents the real radius of the capillary. Image was stretched and strained until the blue line (diameter) was double size of the red line (radius).

6.2.4. Procedures

The study was conducted in a room with controlled temperature (20 to 23°C) and humidity (44% to 53%). PDM and OCT images were taken of the lower tear meniscus of the right eye in primary gaze, directly below the pupil centre in a random order by a single observer. To minimise diurnal and inter-blink variation, measurements were taken in the morning between 10 and 12 o'clock, and 3 to 4 seconds after a normal blink. For both techniques the total measurement time was approximately two minutes, with a break of one minute between the two instruments.

Using ImageJ software, the width of the three bands on the PDM reflected images obtained was measured, and the radius of the meniscus calculated using the concave mirror formula. On the OCT images, the three-point circle fit technique was applied

to calculate the radius (Figure 6.3). In addition, the meniscus on the OCT images was sub-divided vertically into three equal sections and the radius calculated for each sub-section: top (TTMR), centre (CTMR) and bottom (BTMR) (Figure 6.4).

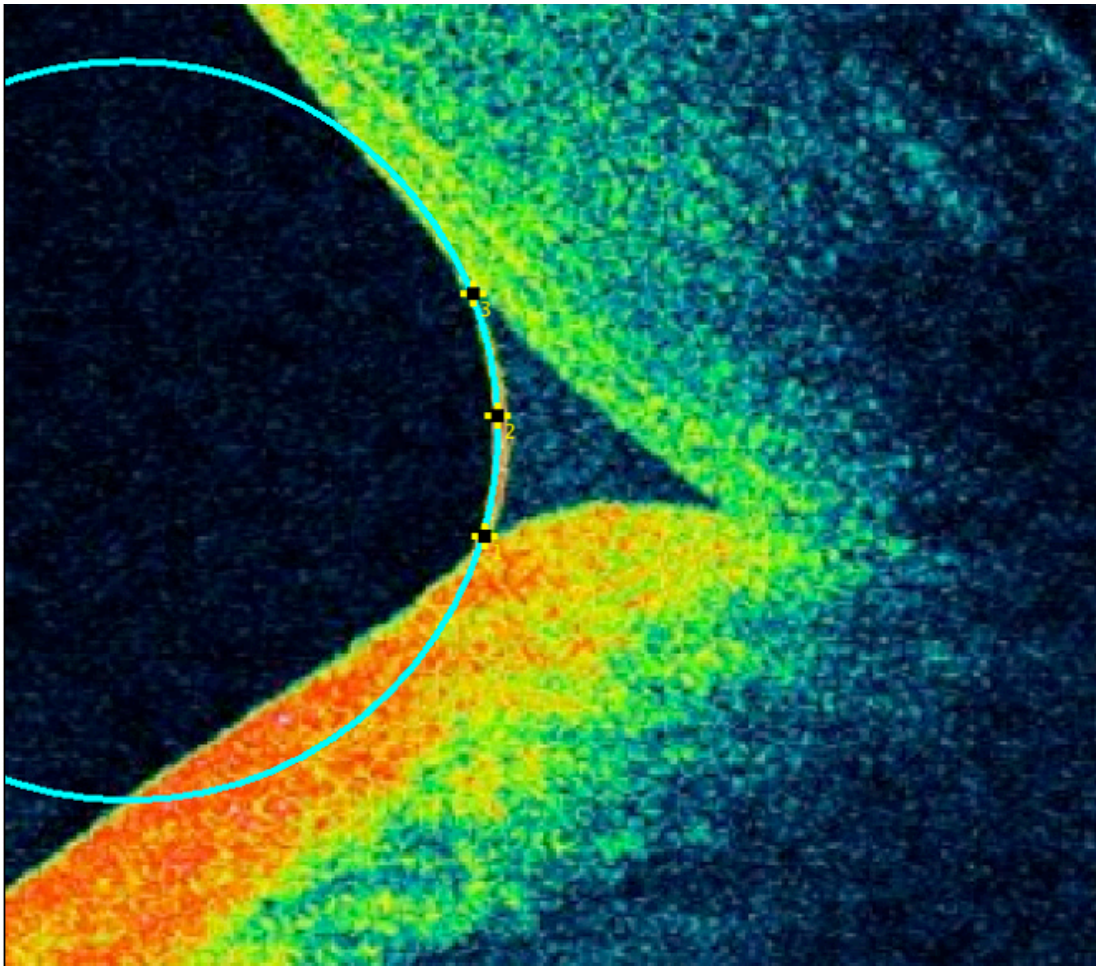


Figure 6.3: Tear meniscus radius measured on the OCT-image, using the 3-point line-fit technique, in ImageJ.

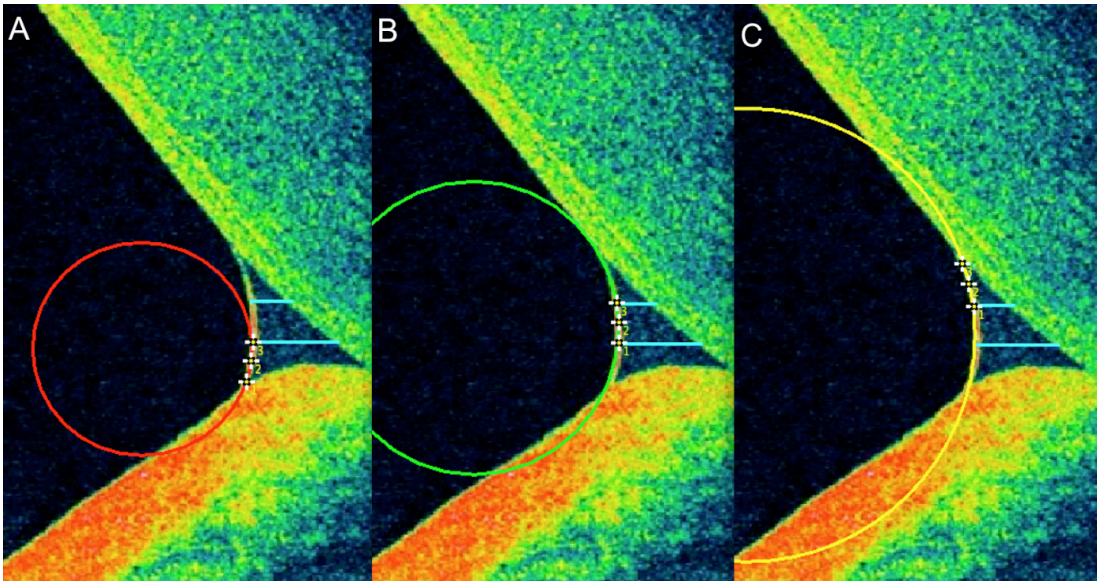


Figure 6.4: Best fit radius for (A) the bottom-section of the tear meniscus (BTMR), for (B) the centre-section of the tear meniscus (CTMR), and for (C) the top-section of the tear meniscus (TTMR).

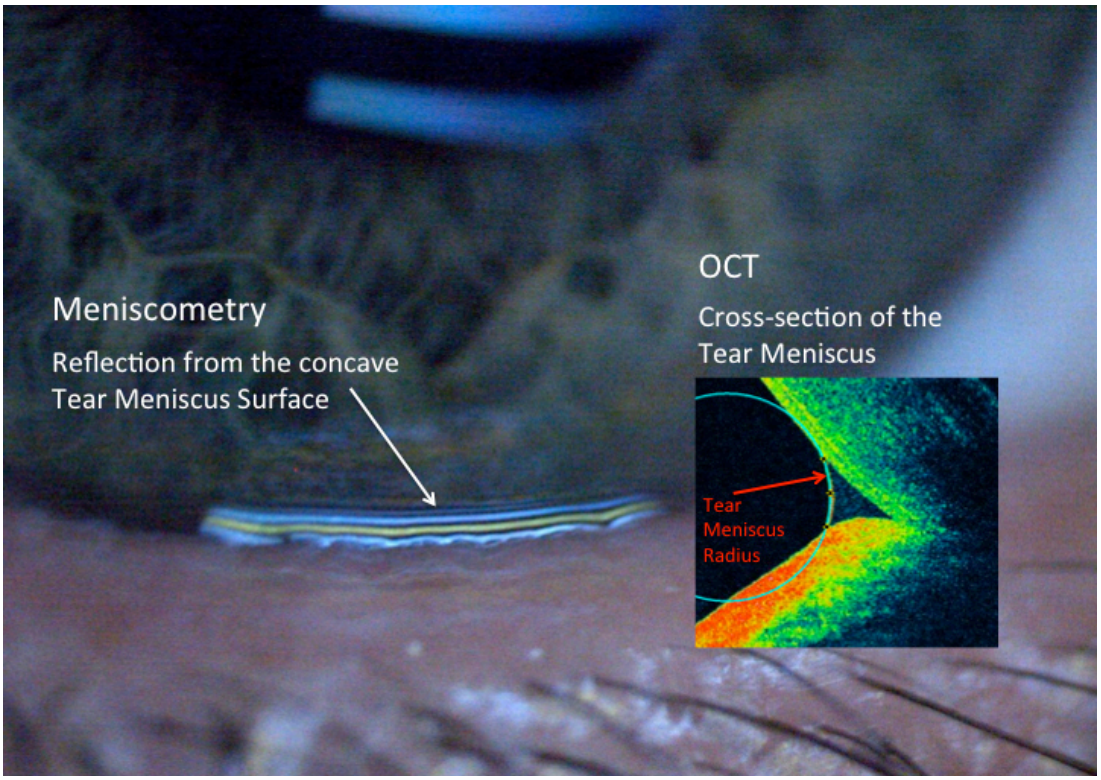


Figure 6.5: Comparison of the two methods applied in this study.

6.3. Statistical analyses

Data were tested for normality using the Shapiro-Wilk test and appropriate statistical tests applied. The data were analysed using SigmaPlot 12 (Systat Software Inc., Chicago, USA) and BiAS 10 (epsilon-Verlag, Darmstadt, Germany). The correlation between PDM and OCT measurements was assessed using Spearman's Rank coefficient, and differences between PDM and OCT sub-section measurements evaluated using paired t-testing and Bland-Altman plots.

6.4. Results

The mean values and standard deviations, and the minimum and maximum values of the lower tear meniscus radius for each of the different measurements, are summarised in Table 6.2. The radii obtained from the sub-sections suggest a parabolic curve for the tear meniscus, where the radius of the upper portion is flatter and becomes progressively steeper in the central and lower portions.

	Mean ± SD [mm]	Minimum [mm]	Maximum [mm]
TMR-PDM	0.25 ± 0.06	0.11	0.36
TMR-OCT	0.29 ± 0.09	0.18	0.56
TTMR-OCT	0.32 ± 0.10	0.16	0.56
CTMR-OCT	0.26 ± 0.09	0.11	0.49
BTMR-OCT	0.18 ± 0.07	0.09	0.39

TMR-PDM, tear meniscus radius measured with PDM; TMR-OCT, tear meniscus radius measured wit OCT; TTMR-OCT, tear meniscus radius measured with OCT in the top-section of the meniscus; CTMR-OCT, tear meniscus radius measured with OCT in the centre-section of the meniscus; BTMR-OCT, tear meniscus radius measured with OCT in the bottom-section of the meniscus.

Table 6.2: Mean ± standard deviation, and minimum and maximum values [mm], of the lower tear meniscus radius for each of the different measurements of central lower tear meniscus radius.

TMR measured with the PDM (0.25±0.06 mm) and OCT (0.29±0.09 mm) was significantly correlated (Spearman's Rank coefficient; $r=0.675$; $p< 0.001$). The mean differences between PDM and sub-sections of the OCT images showed that TMR measured with PDM was similar to that measured in the central region by the OCT (-0.01 mm; CI -0.04 to 0.02; paired t-test; $p=0.636$; Figure 6.6), but was significantly

less for the TTMR (-0.07 mm; CI -0.10 to -0.04; p< 0.001; Figure 6.7), and significantly increased for the BTMR (0.07 mm; CI 0.05 to 0.10; p< 0.001; Figure 6.8). The power calculation of the completed study was 0.95 (α=0.05).

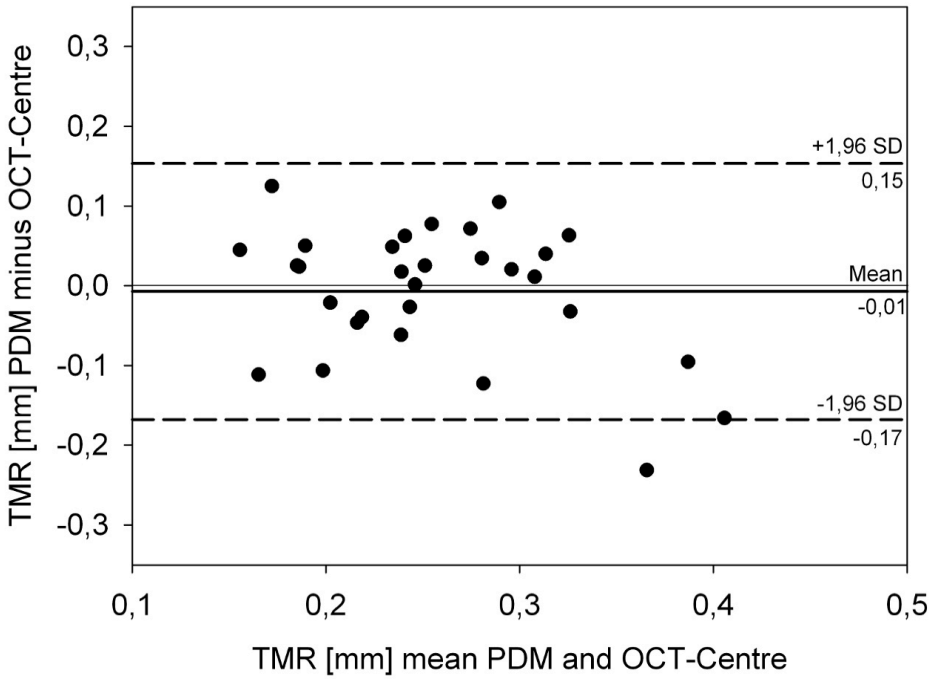


Figure 6.6: Differences between PDM and OCT in the centre-section.

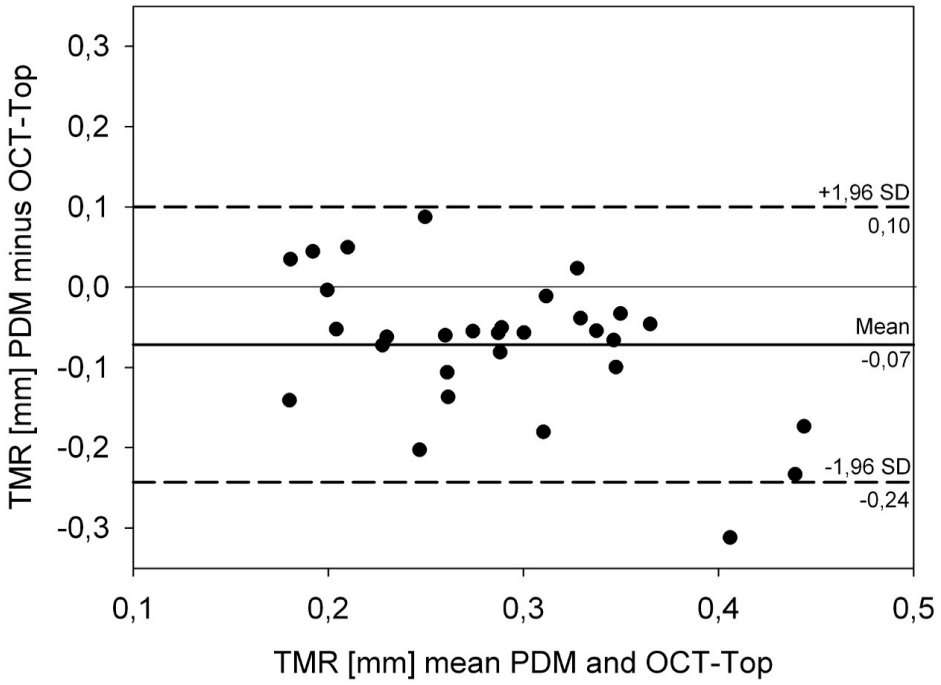


Figure 6.7: Differences between PDM and OCT in the top-section.

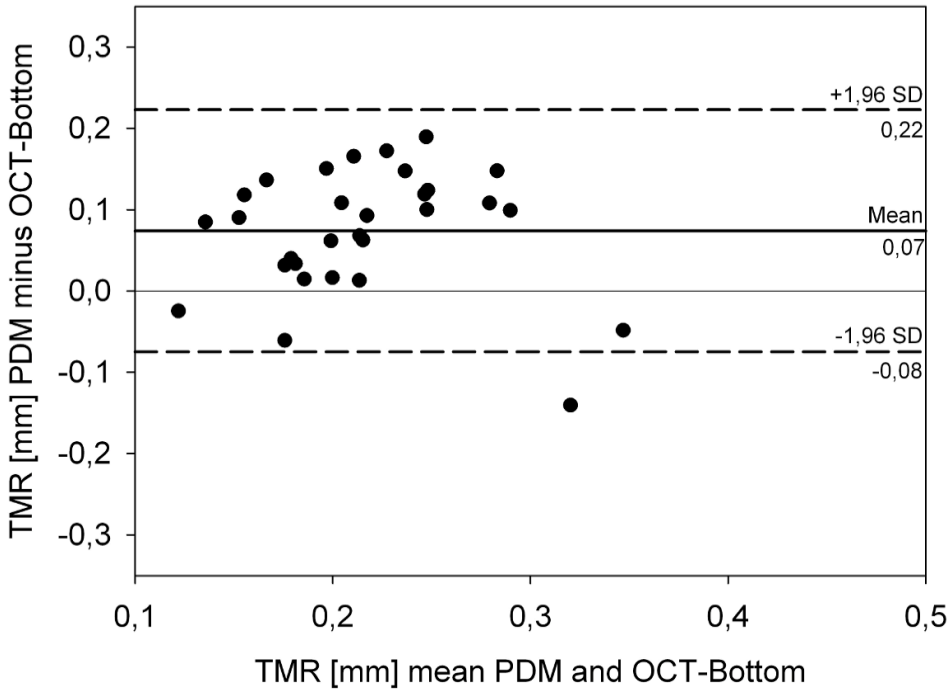


Figure 6.8: Differences between PDM and OCT in the bottom-section.

6.5. Discussion

The PDM is an alternative way to non-invasively measure TMR and this study has demonstrated that it has a high measurement correlation to the existing OCT technique. The PDM therefore has useful potential for TMR measurements that are considered useful in the diagnosis of dry eye, the determination of tear film distribution, and in evaluation of the effectiveness of dry eye treatments.

Using OCT or reflective meniscometry, average TMR values of the lower central meniscus of normal subjects in previous studies have been reported to range from 0.24±0.05 mm to 0.46±0.40 mm (Table 6.1). The results from this study are within this range. This is important since non-invasive measurement of lower TMR has showed good diagnostic accuracy (92% sensitivity and 87% specificity; cut-off value 0.18 mm) in the diagnosis of aqueous-deficient dry eye[28]. In contrast, the average TMR found using the invasive, slit-lamp fluorescein technique ranges from 0.48±0.21 mm to 0.55±0.26 mm (Table 6.1), which gives with a cut-off value of 0.35 mm (80% sensitivity and 87% specificity), as suggested by Mainstone et al.[189].

Kato et al.[351] reported a significant linear correlation between TMR values measured with VM (0.34 ± 0.21 mm) and OCT (0.35 ± 0.26 mm) in a mixed group consisting of 14 normals, 25 dry eye and 14 epiphora subjects. In their study they used the RTVue-100 OCT (Optovue, Fremont, USA), which is also SD-OCT with an axial resolution of 5μm, similar to the OCT used in this study. With the Cirrus HD-OCT we were able to measure TMR by the help of an external image analysing software.

However, there is a significant problem with using the OCT to describe the TMR shape. The dimensions of the images produced by an OCT suffer from distortions in the images paths that cannot be assessed easily. One of these distortions is the "fan-distortion". It is conditioned by the design of the scanner, and the arrangement and design of the mirror and the collimator lens[223, 224], but it has the effect that a flat surface appears to be bent. Further distortions, called "optical distortions", are caused by variations in the refractive indices of the tissue that is being measured[223, 224]. The higher the refractive index of the tissue, the longer the light takes to go through the tissue: this has the result that a measuring scale calibrated to measure corneal thickness, for instance, cannot be used to measure other tissue structures. In order to perform reliable measurements with the OCT despite the resulting distortions, specialist algorithms are required to eliminate these errors[225, 226]. However, such algorithms are part of the OCT software and are not disclosed to the users of the instrument. In this study, the Cirrus HD-OCT (Carl Zeiss Meditec, Jena, Germany) was used. Within its anterior eye module, the ruler measures only vertical distances, with the scale factor calibrated for measuring corneal tissue only. Since the tear meniscus images are produced in air, the ‘in-tissue’ algorithm corrections were no longer appropriate, and so all OCT images were analysed within separate software programs. To calibrate the distances and curvatures on the images, OCT images of glass-capillaries with known radii were used, and then stretched or compressed until no distortions were observed for the first interface. In contrast, there was no need to equalise the PDM images, which made the analysing process easier. The PDM digital images can be directly used and, with the known pixel/mm ratio, distances in all directions can be measured without any transformation.

This study showed that the PDM measures the radius of the central section of the tear meniscus. To our knowledge this is the first OCT study in which the meniscus was sub-divided into three different sections for detailed analysis. As might be expected from a casual perusal of the tear meniscus cross-sections, the steepest TMR was found in the bottom third and the flattest TMR in the top third of the meniscus. In a study by Johnson and Murphy[163], where they used the slit-lamp image capture technique to measure changes in TM after a blink, the TMR was calculated at the top (TMR_t) and at the bottom (TMR_b) of the meniscus. On eye opening, they found (TMR_t) and (TMR_b) to be similar, indicating an approximately circular meniscus profile, while only one second later the radius of the top section was 0.19 mm flatter than that of the bottom section. Thereafter, this difference in radii stabilised.

In this study, the measurements were completed 3 to 4 seconds after a blink and the TMR of the top third was found to be 0.14 mm flatter than that of the bottom third of the meniscus. Although a non-invasive technique was used, and three sub-sections analysed instead of two, their findings of a flatter TMR at the top of the meniscus were confirmed by this study.

During the first 1.5 sec following the blink Johnson and Murphy[163] suggested that TMR increases by about 20%, while others observed the lower TMR to be stable during the inter-blink period[27, 31, 163]. This discrepancy is most likely the result of the different techniques used, or might be due to the observation that only some parts of the meniscus change, while other parts stay stable following a blink[163].

6.6. Conclusions

PDM and OCT measurements of the TMR are significantly correlated. Since with the PDM no image calibration is needed, it seems to be a quick and non-invasive technique for evaluation of tear fluid quantity. The PDM appears to measure the radius of the central section of the tear meniscus.

The published form of this chapter can be found in Appendix 2.2:

Bandlitz S, Purslow C, Murphy PJ, Pult H.

Comparison of a new portable digital meniscometer and optical coherence tomography in tear meniscus radius measurement.

Acta Ophthalmol 2014;92:e112-118.

CHAPTER 7: The Relationship between Tear Meniscus Regularity and Conjunctival Folds

7.1. Introduction

The studies in chapters 5 and 6 have demonstrated that the PDM gives accurate and reliable measurements of TMR at the central position, which are significantly correlated to optical coherence tomography (OCT) and video-meniscometer values[354, 355]. However, the measurements of TMR and the calculation of the cross-sectional area (TMA) are limited to one or, in the case of the area, to two dimensions. Since the meniscus is spread along the eyelid margins, the length of the lid is used to calculate the tear meniscus volume (TMV). As the eyelids are curved, the eyelid length measured on an image is adjusted by a multiplication factor of 1.294, according to Tiffany et al.[241].

In the literature, the measurement of tear meniscus parameters is mostly performed at the centre of the lower eyelid, directly under the pupil. Some authors reported TMH to be greater at the centre of the lid[185], but others analysed no thinning of the inferior tear meniscus[100], or even reported that the TMH is lower at the centre[25]. These differences might be explained by the different techniques used, the timing of such measures after a blink and the different areas of observation. At the same time, when calculating TMV, the meniscus is assumed to be equal along the lower lid[31, 241], or a correction factor of ¾ is used to account for an unequal distribution[177, 185, 356]. Furthermore, the paracentral tear meniscus might be altered by surface abnormalities behind the tear meniscus, like conjunctival folds.

Lid parallel conjunctival folds (LIPCOF) are folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin. LIPCOF were described as a sub-type that might represent a mild stage of conjunctivochalasis[326]. Like conjunctivochalasis, LIPCOFs are located in the tear meniscus area and both are assumed to interfere with the meniscus[329, 332-334].

Chapters 5 and 6 have demonstrated the potential of the PDM to measure the tear meniscus at the central position of the lower eyelid. It is not known how effective this new system is at assessing TMH and TMR at different locations along the lid margin, in order to describe the distribution of tear fluid along the lower eyelid.

The aims of this study are: (i) to investigate the capability of the new slit-lamp mounted PDM to measure TMH and, for the first time, TMR at different locations along the lower lid; and (ii) to evaluate any relationships between tear meniscus regularity and the degree of LIPCOF.

7.2. Methods

7.2.1. Subjects

Forty-two subjects (male = 13, female = 29) were randomly selected from the staff and students of the Höhere Fachschule für Augenoptik Köln (Cologne School of Optometry), Cologne, Germany. Subjects were excluded if they were pregnant or breast-feeding; had a current or previous condition known to affect the ocular surface or tear film; had a history of previous ocular surgery, including refractive surgery, eyelid tattooing, eyelid surgery, or corneal surgery; had any previous ocular trauma,

were diabetic, were taking medication known to affect the ocular surface and/or tear film. Since contact lens wear was shown to influence the tear meniscus and LIPCOF grade[332], all subjects were not allowed to wear contact lenses during, and two weeks prior to, the study.

Each subject's symptoms were evaluated using the Ocular Surface Disease Index (OSDI) questionnaire and afterwards the total OSDI scores were calculated[252]. The subjects were then classified into symptomatic (OSDI score ≥ 13) and asymptomatic patients (OSDI score < 13)[357].

All subjects gave written informed consent before participating in the study. All procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Research Audit Ethics Committee and were conducted in accordance with the requirements of the Declaration of Helsinki.

7.2.2. Instrumentation and procedures

The newly developed, slit-lamp mounted, portable, digital meniscometer (PDM) was used to measure TMH and TMR along the lower eyelid. The detailed construction of the PDM has been described in Chapter 4.2.[354, 355]. The PDM is mounted on a metal axis and fixed to the tonometer post of the slit-lamp and therefore the target can be rotated to avoid shadowing caused by the nose. Using the PDM, TMH and TMR was measured in a randomised order at three locations along the lower lid of one eye: central, perpendicularly below the pupil centre (TMR-C; TMH-C); and temporal (TMR-T; TMH-T) and nasal (TMR-N; TMH-N), perpendicularly below the limbus

(Figure 7.1). An earlier study showed that the PDM measures the radius of the central section of the tear meniscus[355]. The anterior surface of the tear meniscus was found to have a parabolic shape[358], and PDM measurement of the central section of the tear meniscus was found to be in good agreement with the OCT 3-point line-fit technique, where the bottom, centre and upper boundaries of the anterior meniscus surface were delineated[355].

To minimise diurnal and inter-blink variation, images were recorded in the morning between 10 and 12 o'clock, and 3 to 4 seconds after a normal blink.

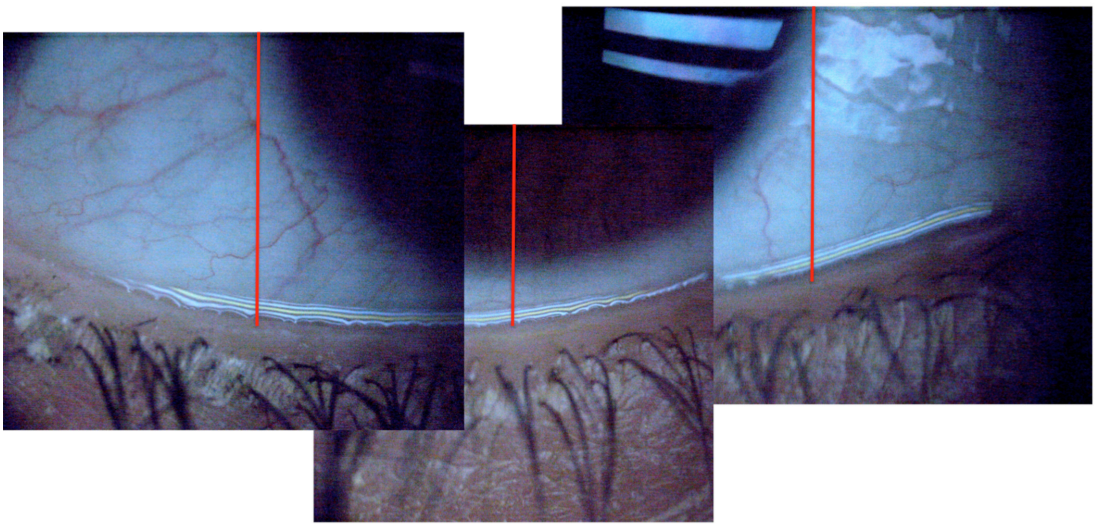


Figure 7.1: Reflected image of the portable digital meniscometer (PDM) lines on the concave temporal, central, and nasal tear meniscus. The picture is a composition of three single slit-lamp images with the red line marking the measuring location.

Lid-parallel conjunctival folds were evaluated without fluorescein using a slit-lamp microscope (BQ900, Haag-Streit, Koeniz, Switzerland) and 25x magnification (Figure 7.2). LIPCOF were observed perpendicular from the temporal and nasal limbus down to the lower lid margin, which were the same locations at which the TMH and TMR were measured. LIPCOF was classified using the optimised grading scale (Table 7.1)[327, 359]. Care was taken to differentiate LICPOF from micro-folds, which are less well organised and around three times smaller than LIPCOF[331].

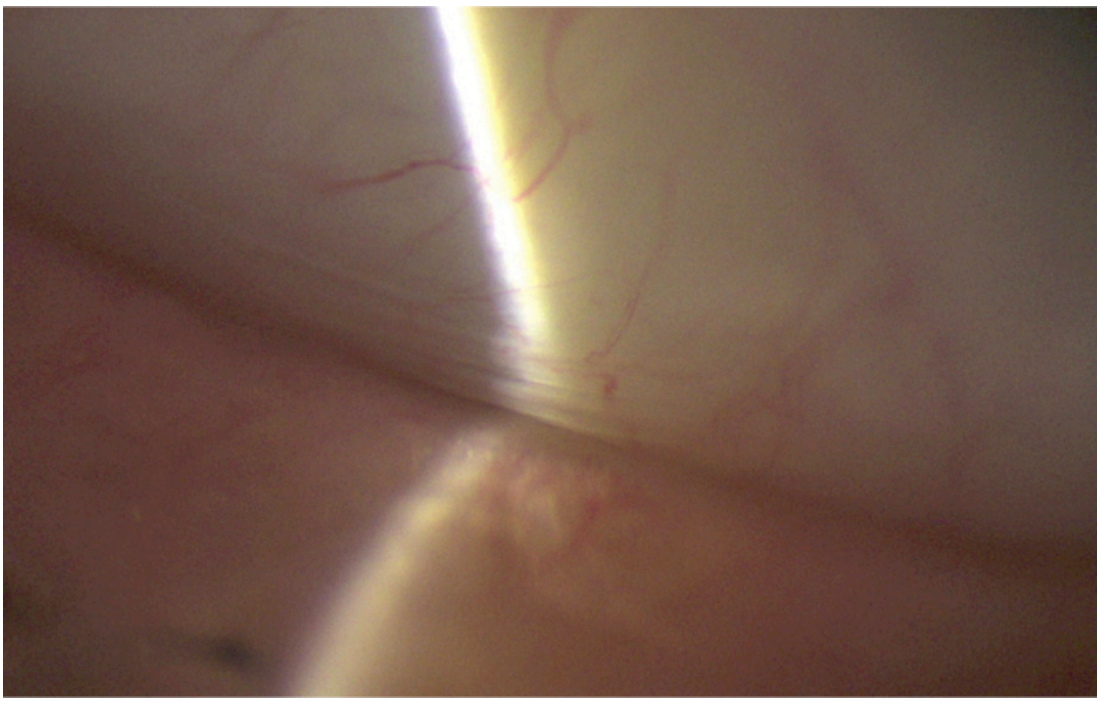


Figure 7.2: Real slit-lamp image of lid parallel conjunctival folds (LIPCOF) grade 3 at the temporal position.

LIPCOF Grade	
0	No conjunctival folds
1	One permanent and clear parallel fold
2	Two permanent and clear parallel folds, (normally <0.2 mm)
3	More than two permanent and clear parallel folds, (normally >0.2 mm)

Table 7.1: Optimised grading scale of lid-parallel conjunctival fold (from Berry et al.,2008)[327].

The study was conducted in a room with controlled temperature (20 to 23°C) and humidity (44 to 53%). All lower tear meniscus measurements and LIPCOF evaluations were taken on the right eye in primary gaze controlled by a fixation target in a randomised order by a single observer. Analysis of tear meniscus parameters was masked against LIPCOF grading.

7.3. Statistical analyses

Data were tested for normality using the Shapiro-Wilk test and appropriate statistical tests applied. Correlations were analysed with Pearson correlation (or Spearman rank in non-parametric data). The differences between the locations along the lower lid were calculated with a paired t –test. To detect the differences among the LIPCOF-groups, one-way ANOVA and post-hoc Fisher Least Significant Difference (LSD)

tests were used ($p<0.05$). The data were analysed using SigmaPlot 12 (Systat Software Inc., Chicago, USA).

7.4. Results

7.4.1. Regularity of Tear Meniscus Height

TMH-C (0.20 ± 0.04 mm) was significantly correlated to TMH-T (0.27 ± 0.07 mm; Pearson; $r=0.561$, $p<0.001$) and TMH-N (0.25 ± 0.06 mm; $r=0.529$, $p<0.001$). TMH-T (0.063 ± 0.061 mm, $p<0.001$) and TMH-N (0.046 ± 0.044 mm, $p<0.001$) were both significantly higher than TMH-C (Figure 7.3). However, no significant differences were found between TMH-T and TMH-N ($p=0.118$).

7.4.2. Regularity of Tear Meniscus Radius

TMR-C (0.27 ± 0.08 mm) was significantly correlated to TMR-T (0.31 ± 0.10 mm; Pearson; $r=0.653$) and TMR-N (0.30 ± 0.11 mm; $r=0.770$) ($p<0.001$). TMR-T (0.041 ± 0.082 mm, $p=0.002$) and TMR-N (0.026 ± 0.076 mm, $p=0.038$) were both significantly flatter than TMR-C (Figure 7.4). No significant differences were found between TMR-T and TMR-N ($p=0.159$).

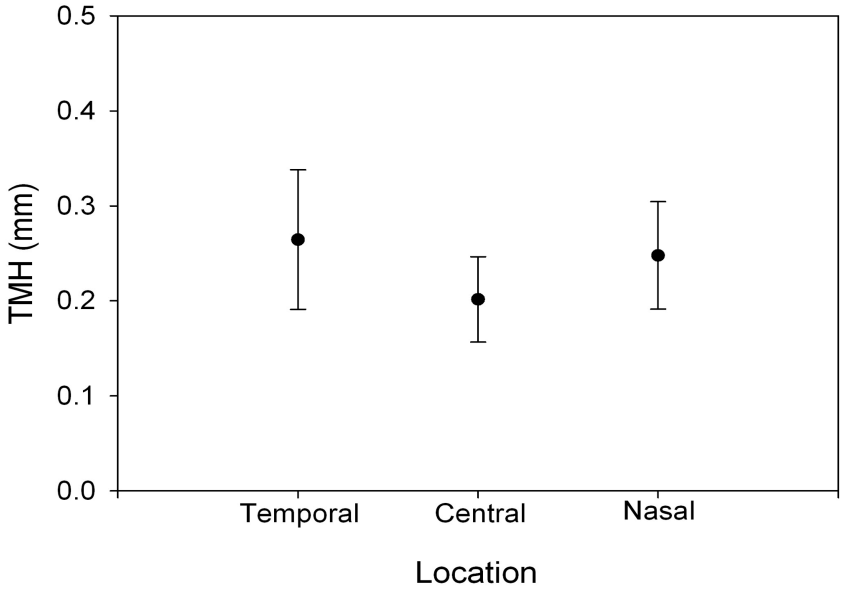


Figure 7.3: Mean ± standard deviation of tear meniscus height at the temporal, central and nasal positions of the lower eye-lid.

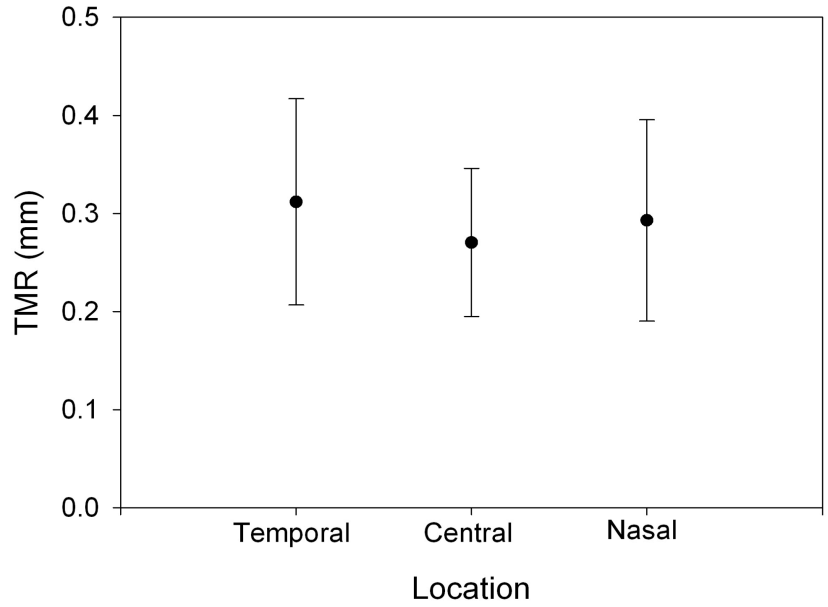


Figure 7.4: Mean ± standard deviation of tear meniscus radius at the temporal, central and nasal positions of the lower eye-lid.

7.4.3. Relationship between LIPCOF Grades and Tear Meniscus Regularity

Temporal LIPCOF scores (1.43 ± 0.86) were significantly correlated to nasal LIPCOF scores (0.57 ± 0.79) (Spearmans Rank; r=0.317; p<0.05). Temporal LIPCOF scores were significantly correlated to the difference between TMH-T and TMH-C (r=0.590; p<0.001) (Figure 7.5) and to the difference between TMR-T and TMR-C (r=0.530; p<0.001) (Figure 7.6), while nasal LIPCOF scores were significantly correlated to the difference between TMH-N and TMH-C (r=0.492; p=0.001) (Figure 7.7) and to the difference between TMR-N and TMR-C (r=0.350; p=0.023) (Figure 7.8). The power calculation of the completed study resulted in a power >0.86 (α=0.05).

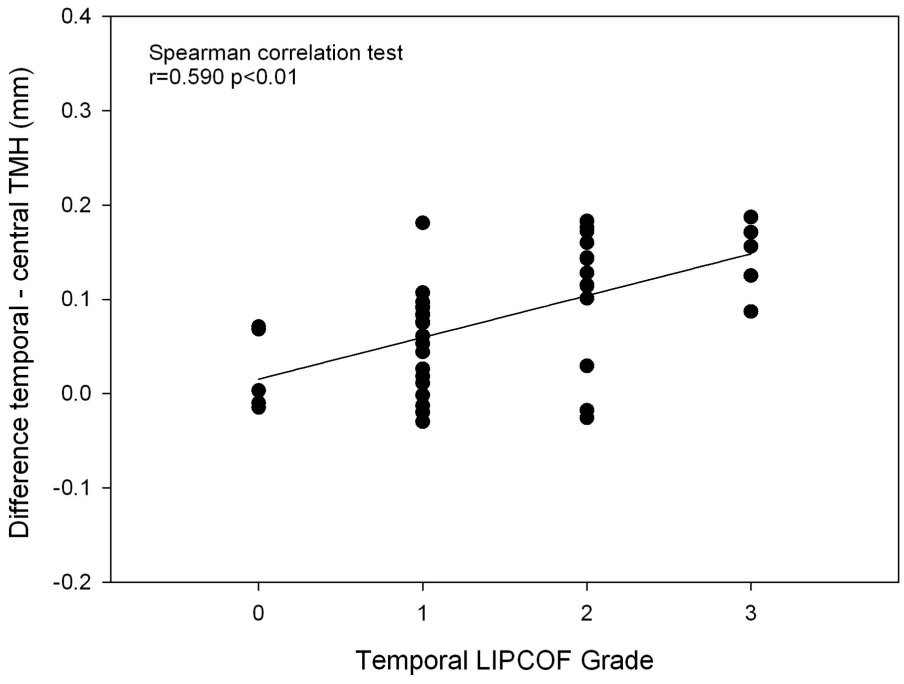


Figure 7.5: Correlation between temporal LIPCOF grades and change in temporal tear meniscus height (TMH).

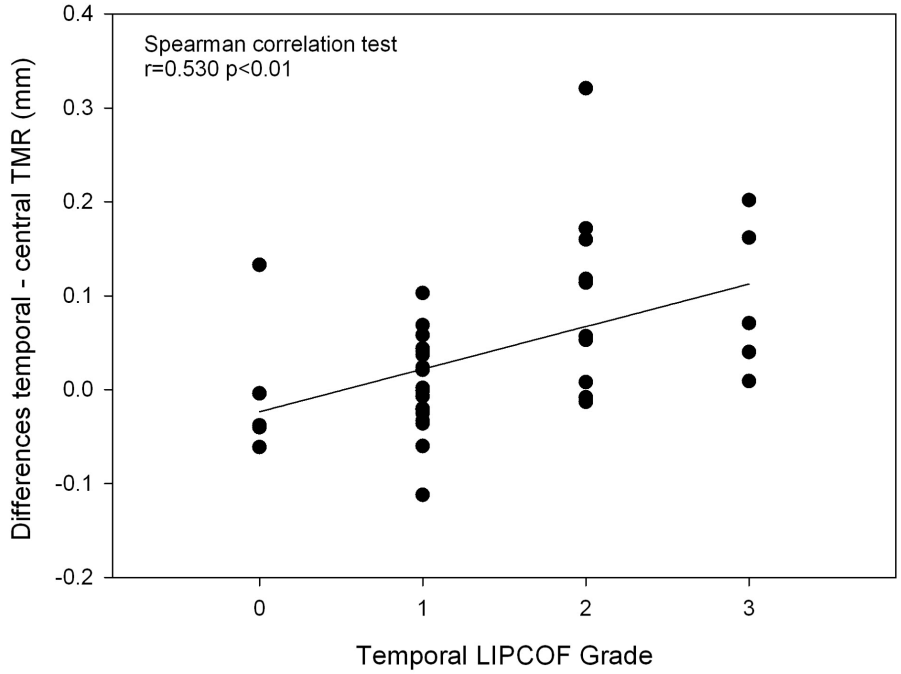


Figure 7.6: Correlation between temporal LIPCOF grades and change in temporal tear meniscus radius (TMR).

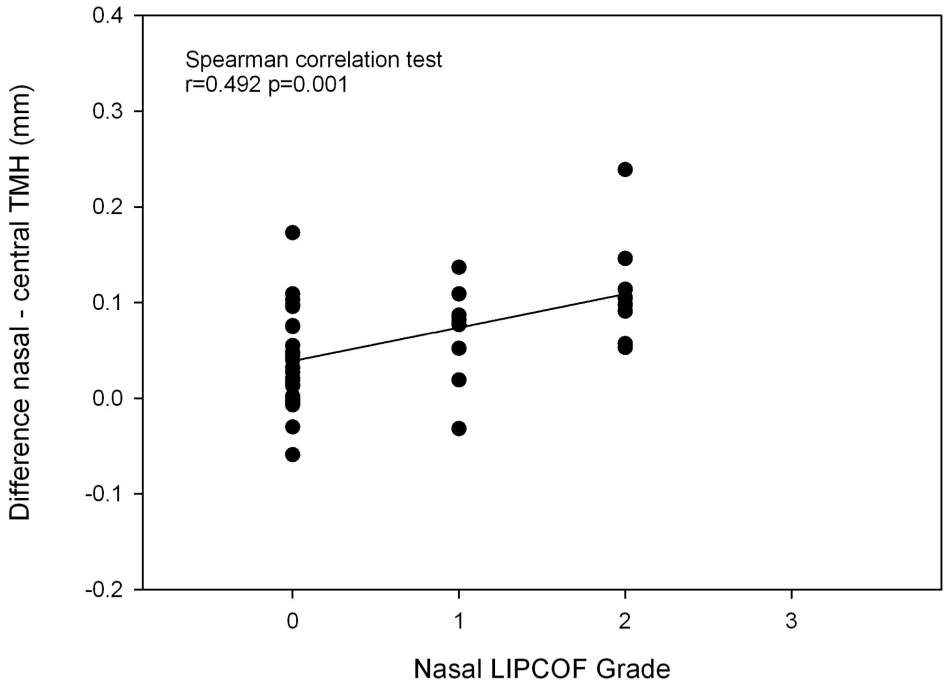


Figure 7.7: Correlation between nasal LIPCOF grades and change in nasal tear meniscus height (TMH).

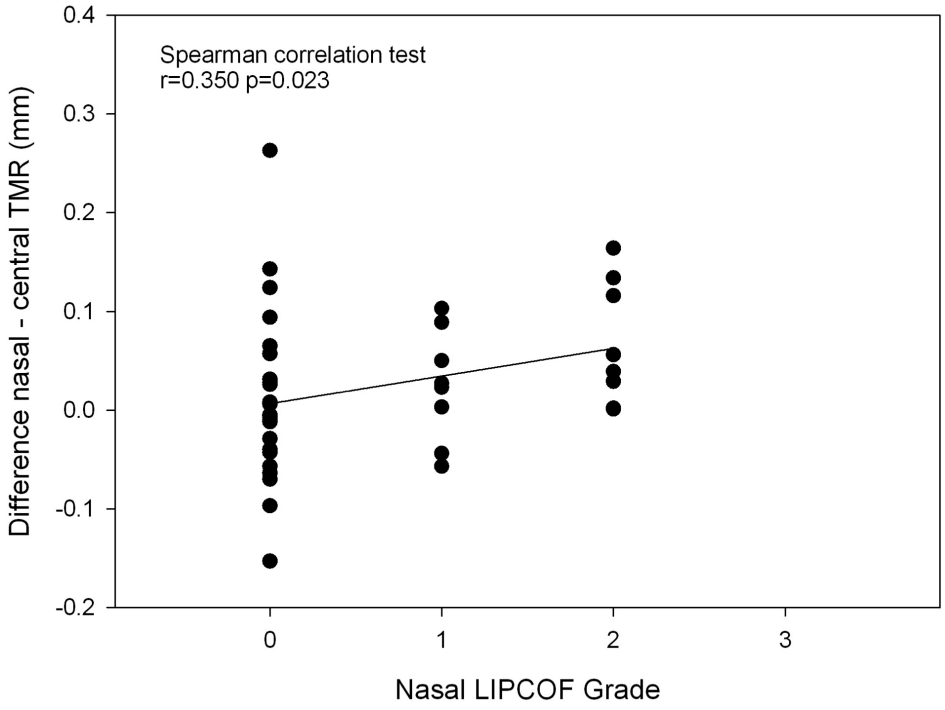


Figure 7.8: Correlation between nasal LIPCOF grades and change in nasal tear meniscus radius (TMR).

With temporal LIPCOF grades of ≤1, the temporal TMH and TMR were similar to the central TMH and TMR, while for LIPCOF grades ≥2 they were significantly different (Figures 7.9 and 7.10). Similarly, for the nasal LIPCOF grades of ≤1, the nasal TMH and TMR were not different from the central TMH and TMR, but were significantly different for LIPCOF grades of 2 compared to grade 0 (Figure 7.11 and 7.12).

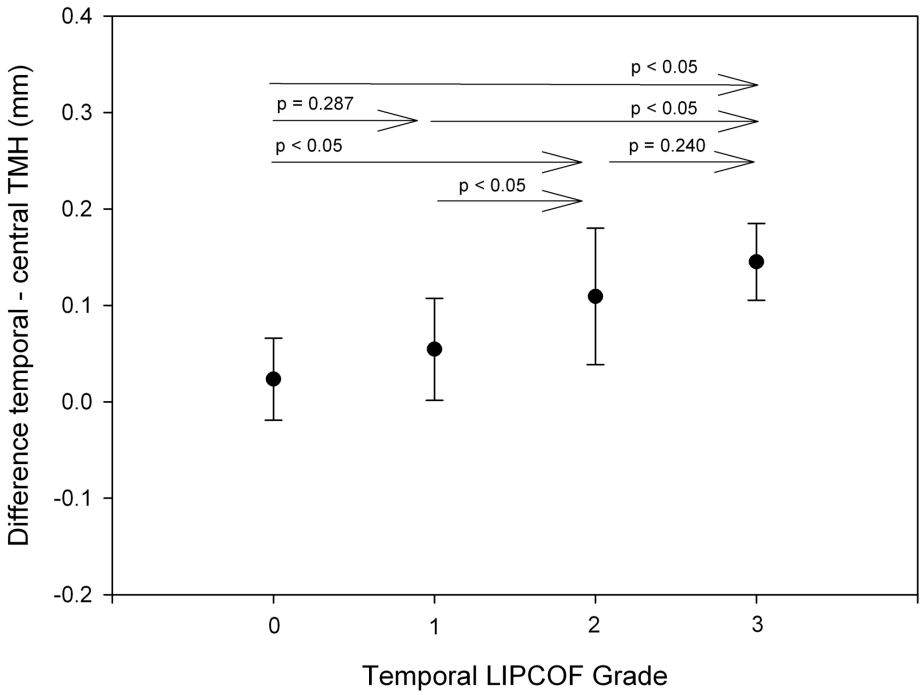


Figure 7.9: Mean difference between the temporal and central tear meniscus heights in the four sub-groups, across different lid-parallel conjunctival folds grades.

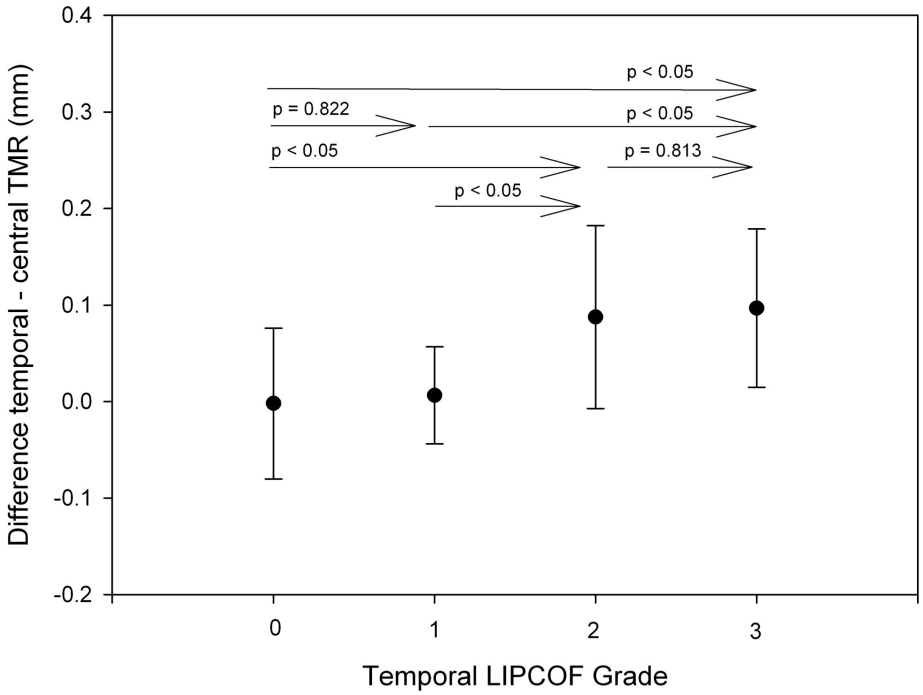


Figure 7.10: Mean difference between the temporal and central tear meniscus radii in the four sub-groups, across different lid-parallel conjunctival folds grades.

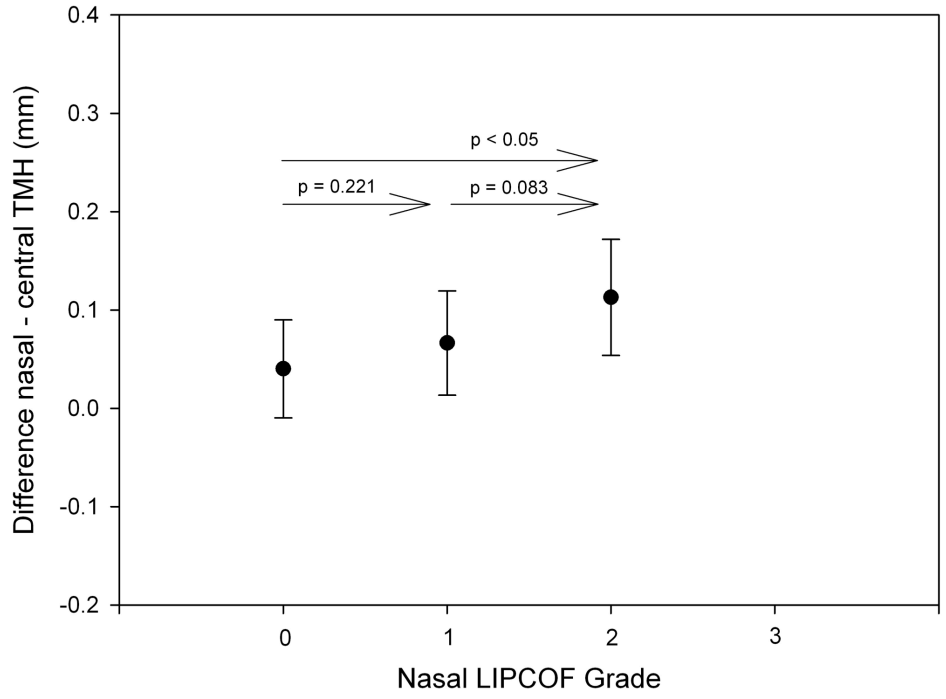


Figure 7.11: Mean difference between the nasal and central tear meniscus heights in the four sub-groups, across different lid-parallel conjunctival folds grades.

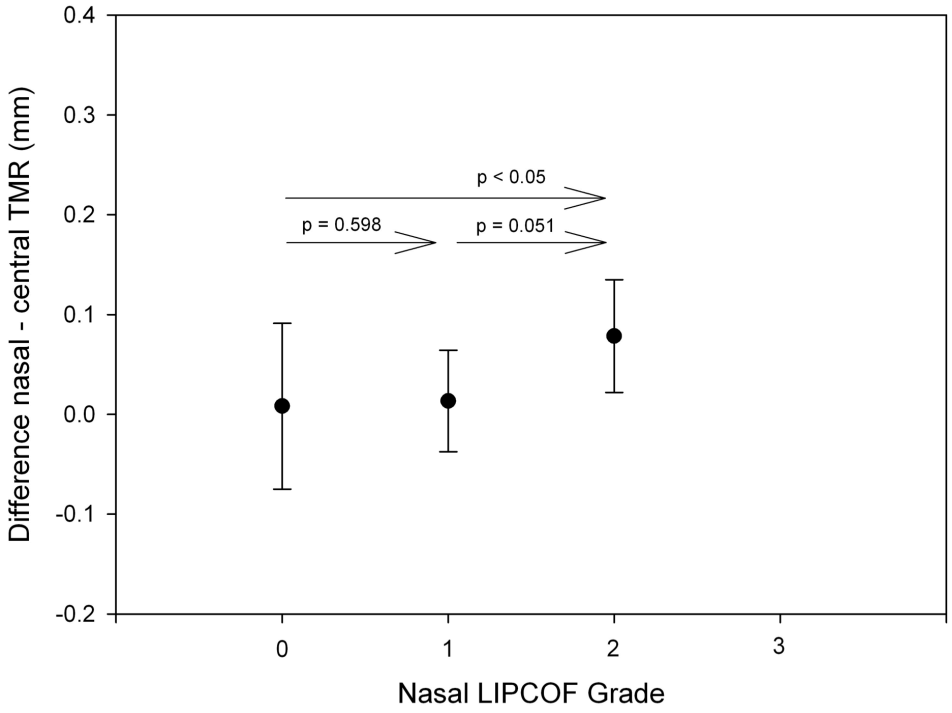


Figure Figure 7.12: Mean difference between the nasal and central tear meniscus radii in the four sub-groups, across different lid-parallel conjunctival folds grades.

7.4.4. Dry Eye Symptoms and LIPCOF grade

Mean OSDI score was 10.7 ±7.3 (SD) with a range from 0 to 32.5. The OSDI scores LIPCOF grades, TMH and TMR for the asymptomatic and symptomatic subjects are summarised in Table 7.2. There was a statistically significant difference (p=0.039) in temporal LIPCOF grades between the asymptomatic and symptomatic subjects, while there was no statistically difference (p=0.964) for the nasal LIPCOF grades.

	Asymptomatic (n=26)	**Symptomatic (n= 16)**
	Mean ±SD or number	Mean ±SD or number
OSDI Score	5.99 ±3.61	18.26 ±5.28 *
Temporal LIPCOF Grade	1.16 ±0.73	1.81 ±0.91 *
Nasal LIPCOF Grade	0.58 ±0.81	0.56 ±0.81
Temporal LIPCOF (grad 0/grad 1/grad 2/grad 3)	(5/12/9/0)	(0/8/3/5)
Nasal LIPCOF (grad 0/grad 1/grad 2/grad 3)	(16/5/5/0)	(10/3/3/0)
Central TMH	0.20 ± 0.05 mm	0.21 ± 0.04 mm
Temporal TMH	0.26 ± 0.07 mm	0.27 ± 0.09 mm
Nasal TMH	0.25 ± 0.06 mm	0.24 ± 0.06 mm
Central TMR	0.26 ± 0.08 mm	0.29 ± 0.07 mm
Temporal TMR	0.31 ± 0.10 mm	0.32 ± 0.11 mm
Nasal TMR	0.28 ± 0.09 mm	0.32 ± 0.12 mm

Table 7.2: OSDI scores, LIPCOF grades, TMH and TMR for the asymptomatic and symptomatic subjects. Asterisk indicates a statistically significant difference ($p < 0.05$).

7.5. Discussion

This study has found that the PDM is able to measure TMH and TMR at different locations along the lower lid. The results for the central TMH and TMR were within the range of previous values reported for central TMH: 0.10 ± 0.04 mm to 0.46 ± 0.17 mm, and central TMR from 0.15 ± 0.03 mm to 0.55 ± 0.26 mm[189, 193-195, 240, 344].

Temporal and nasal TMH were significantly higher than central TMH. This is in agreement with the observation of Garcia-Resua et al.[25], even though they reported slightly lower values. However, they measured TMH as the distance between the darker edge of the lower eyelid and the upper limit of the brightest reflex of the meniscus, while in this study the upper limit of the tear meniscus was measured. However, identifying the upper limit of the meniscus at the slit lamp is challenging unless sodium fluorescein is added to the tear film, which in turn renders the test invasive and will introduce errors. In contrast, TMR measurement is non-invasive and since the radius is measured, there is no need to detect the upper limit of the meniscus.

The PDM was also able to measure TMR at different locations along the lower lid. To our best knowledge it was for the first time TMR was measured at different locations. In previous studies a significant positive correlation has been reported between TMH and TMR at the central position, thus a steeper TMR can be expected in eyes with lower TMH, while a flatter TMR correlates with higher TMH[37, 203]. In this study, a flatter TMR was found at the temporal and nasal position compared to the central position, which concurred with the higher values of TMH at these locations.

In contrast to these findings, Jones at al.[185] reported that central TMH was significantly greater than that found in the nasal and temporal areas 3 mm from the nasal and temporal canthi. These differences may be principally explained by the different locations between the two studies. Furthermore, in this study the measuring time after a blink was controlled (3-4 sec after a blink) while it was not controlled in the study by Jones et al.[185] However, Maki et al.[360, 361] has shown that, based on a mathematical model, the volume distribution of the tear film changes significantly over time between blinks. Jones at al.[185] hypothesised that gravity forces a pool of tears to form at the centre of the lower eye lid, while Garcia-Resua at al.[25] hypothesised that tear fluid surface tension may explain the higher values of nasal and temporal TMH.

Harrison et al.[100] showed no significant thinning of the inferior tear meniscus at the limbus compared to the central cornea. However, since they visualised the meniscus with fluorescein and also measured TMH at the area where the lower lid contacts the limbus, it is inappropriate to compare their results with our findings.

Observed temporal and nasal LIPCOF degrees in this study are in concordance with previously reported LIPCOF[165, 332, 359]. LIPCOF scores have been reported to be increased in dry eye, but they are not age-related[325, 328], while conjunctivochalasis has been defined as the redundant, loose, non-oedematous conjunctival tissue found at the lower eyelid, typically in older people[329, 330]. The temporal LIPCOF score in this study was greater in the symptomatic group, which supports earlier findings of LIPCOF being a good discriminator between normal and dry eye patients[198, 362].

Since LIPCOF and conjunctivochalasis are both located in the area of the tear meniscus it is possible that they can influence the distribution of tear fluid along the lower eyelid. Huang et al.[334] found that the conjunctival folds in conjunctivochalasis obliterate tears not only in the meniscus, but also in the reservoir, and they assumed that the conjunctival folds could occupy and deplete the tear reservoir in the fornix. Conjunctivochalasis is often used to describe more prominent folds than described by LIPCOF, being around 0.08 mm height[331].

The severity of conjunctival folds can be affected by the status of contact lens wear. This effect is thought to be an immediate mechanical effect of the contact lens,[332] or a long-term effect caused by an increased friction due to tear film instability[359]. While in this study the subjects were not allowed to wear contact lenses during the procedure and for two weeks before the study, an immediate effect can be negated. It is possible that a long-term effect of contact lens wear might have influenced the LIPCOF grades.

Using OCT images, Veres et al.[363] observed the coverage of LIPCOF by the tear meniscus and hypothesised that after a blink there is a coverage of the conjunctival folds by the tear film. However, in this study an irregularity of TMH and TMR was found with LIPCOF grades 2 and 3. Therefore one hypothesis may be that LIPCOF in the tear meniscus act as a barrier to the normal flow of tears along the lower eyelid (tear flows along the lower lid margin from temporal side towards the punctum and takes about 3 sec after blink[83, 100]), and that this impedance to tear flow produces an increase in tear volume at the temporal and nasal location of the LIPCOFs (Figure 7.13). A similar idea was previously described by Guillon[243]. He argued that

LIPCOF might affect the morphology of the reservoir so that it loses its meniscus shape and follows the contour of the underlying conjunctiva.

Holly and Lemp[39] reported that a scanty or discontinuous inferior tear meniscus was indicative of an aqueous tear deficiency or lipid abnormality. Taylor[242] described the inferior tear meniscus as "intact", "not intact temporally" or "not intact" and found the marginal tear strip continuity to be a method of assessing the adequacy of the tear film. Guillon[243] reported that the reservoir may be interrupted and that this is one sign of potential dry eye symptoms. A subjective classification of tear meniscus profile was suggested by Khurana et al.[244] and modified by Garcia-Resua et al.[25]. Grades 1 and 2 represent a healthy meniscus, whereas grades 3 and 4 represent an abnormal meniscus.

When comparing the change in central lower TMH immediately after a voluntary blink with TMH 3 seconds after the blink, Veres et al.[363] observed an almost 10-fold higher central tear volume decrease in patients with multiple conjunctival folds than in patients with single folds. They assumed that a sharp decrease in tear volume occurs after blinking in the area of the multiple folds. This seems to agree with the findings in this study showing that in the presence of LIPCOF scores greater than one a smaller central TM is produced, compared to temporal or nasal TM, when measurement was performed 3-4 seconds after a blink. On the basis of this we can speculate that, following a blink, the tear flow may be driven from the central to the temporal and nasal LIPCOF areas, leading to a central decrease and temporal/nasal increase of TM. It may be hypothesised that the small distance between two conjunctival folds generates sufficient capillary force to draw tear fluid towards the

folds (Figure 7.14). This force might be more strongly generated if there is more than one fold, which would explain the alteration in TM with LIPCOF grades of ≥ 2, as analysed in this study.

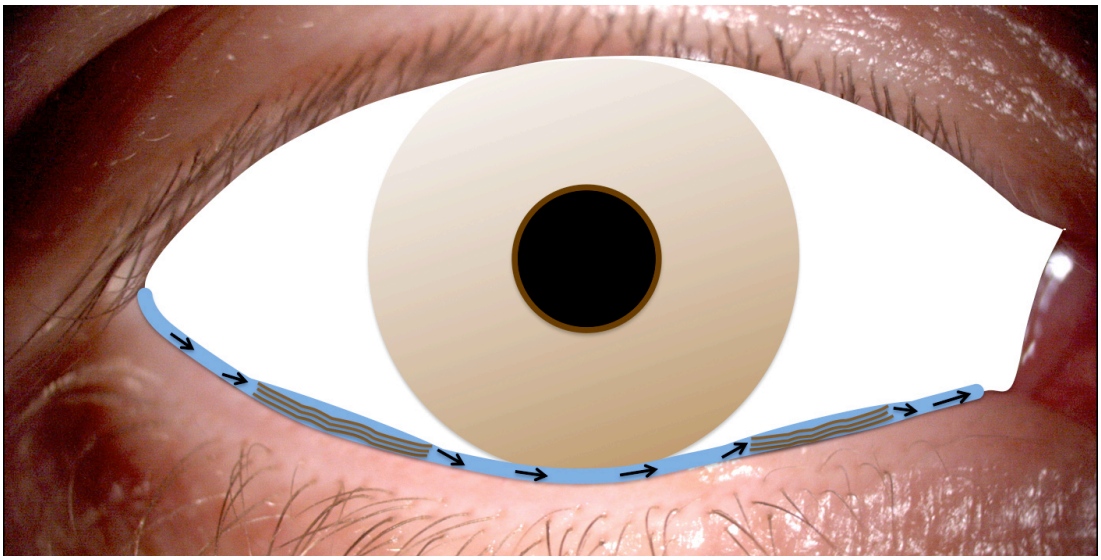


Figure 7.13: Barrier hypothesis for an irregular tear meniscus along the lower lid: The lid-parallel conjunctival folds in the tear meniscus act as a barrier, and tear flow from the outer to the inner canthus is impounded at the temporal and nasal location of the folds.

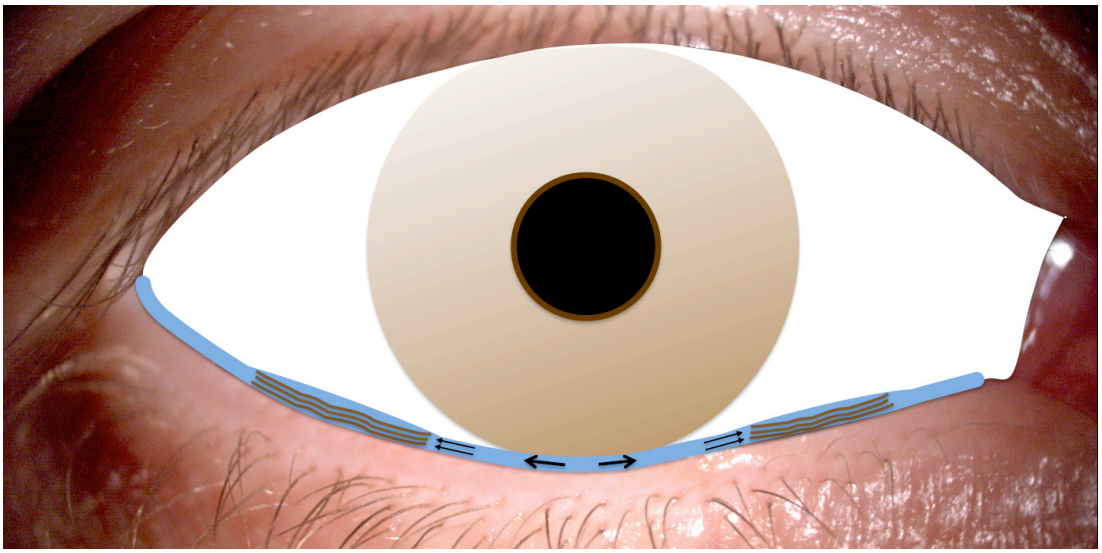


Figure 7.14: Capillary hypothesis for an irregular tear meniscus along the lower lid: The small distance between two lid-parallel conjunctival folds generates capillary forces that draw the surrounding tear fluid towards the folds after a blink.

7.6. Conclusions

In summary, the PDM is able to non-invasively measure alterations in TMR and TMH along the lower lid. The flatter TMR and higher TMH at the nasal and temporal locations may be caused by the LIPCOF degree of the underlying conjunctiva. To avoid any interference by LIPCOF, it is recommended that TMR and TMH are measured along the lower lid margin below the pupil center.

The published form of this chapter can be found in Appendix 2.3:

Bandlitz S, Purslow C, Murphy PJ, Pult H.

The Relationship between Tear Meniscus Regularity and Conjunctival Folds.

Optom Vis Sci 2014;91:1037-1044.

CHAPTER 8: Time Course of Changes in Tear Meniscus Radius and Blink Rate after Instillation of Artificial Tears

8.1. Introduction

Tear fluid produced by the secretory system is distributed and mixed with the pre-ocular tear film and menisci with each blink and then lost by evaporation, absorption and drainage from the menisci through the nasolacrimal passage[83]. Normal tear film dynamics requires a balance between production and elimination of tears from the eye[82]. The production by the lacrimal secretory rate is correlated with tear volume, and the measurement of tear meniscus radius (TMR) is related to tear volume[29, 62]. Blinking is important for the distribution and for the drainage of the tear fluid[31, 157, 364]. The blink rate is influenced by various factors, such as ocular irritation, pre-corneal tear film condition, visual demands, or environmental conditions[159, 161, 162].

Artificial tears are commonly used to increase tear volume and retention, and to improve tear film quality. The retention time of instilled fluids like artificial tears has been studied with different techniques like dacryoscintigraphy, reflective meniscometry, or optical coherence tomograhphy[26, 177, 238, 365, 366]. However, the impact of different solutions on the time course of changes in blink rate and simultaneously on the change in tear volume remains unknown.

In the chapters 5 and 6, the Portable Digital Meniscometer (PDM) has been demonstrated to give accurate and reliable measurements of TMR at a central position, which were significantly correlated to optical coherence tomography (OCT)

and video-meniscometer values[354, 355]. Furthermore, the PDM has shown the capability to detect variations in TMR along the lower lid (Chapter 7)[367]. However, it is not known how effective this new system is at assessing TMR changes after the instillation of artificial tears.

The aims of this study were: (i) to investigate the capability of the portable digital meniscometer (PDM) to measure alterations in TMR after the instillation of artificial tears and (ii) to evaluate any relationships between TMR alterations and changes in blink rate.

8.2. Methods

8.2.1. Subjects

Twenty-two healthy subjects (mean age 24.3 ± 2.6 (SD) years, male = 11, female = 11) were recruited from the staff and students of the Höhere Fachschule für Augenoptik Köln (Cologne School of Optometry), Cologne, Germany. Subjects were excluded if they were pregnant or breast-feeding; had a current or previous condition known to affect the ocular surface or tear film; had a history of previous ocular surgery, including refractive surgery, eyelid tattooing, eyelid surgery, or corneal surgery; had any previous ocular trauma, were diabetic, were taking medication known to affect the ocular surface and/or tear film, and/or had worn contact lenses during the preceding two weeks prior to the study. All subjects gave written informed consent before participating in the study. The procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Research Audit

Ethics Committee and were conducted in accordance with the requirements of the Declaration of Helsinki.

8.2.2. Instrumentation and procedures

Ocular Surface Disease Index

Each subject's symptoms were evaluated before the application of the drop using the Ocular Surface Disease Index (OSDI) questionnaire and afterwards the total OSDI scores were calculated[252]. Analysis of OSDI was masked against tear meniscus and blink rate measurements.

Tear Meniscus Radius Measurement

The slit-lamp mounted portable digital meniscometer (PDM) was used to measure the central TMR at the lower eyelid. The detailed construction of the PDM has been described in Chapter 4.2[354, 355].

Using the PDM and digital slit-lamp, the tear meniscus was videoed over a period of 30 seconds at baseline and 0, 1, 5, 10, and 30 minutes after instillation of either an artificial tear containing hydroxypropyl-guar and glycol (Systane Balance® (SYS), (Alcon Laboratories, Inc., Fort Worth, USA) with a viscosity of 42 cP, or an isotonic sodium chloride solution (SAL) (Lens Plus OcuPure, Abbott Medical Optics Inc., Santa Ana, USA), viscosity 1 cp. Using a micropipette (Pipetman®, Gilson S.A.S., Villiers-le-Bel, France), a defined drop size of 35µl was applied in the temporal lower fornix of the right eye. This drop size represents an average of ophthalmic solution drop sizes[368, 369], and was previously used in similar studies[164, 177, 238, 365]. The drops were applied in a randomised order with a wash-out period of at least one

week between the different solutions. Care was taken to avoid overspill when applying the drop. An image for analysis, at each time point, was captured from the recorded video of the meniscus two seconds after a spontaneous blink when a stable image was achieved. The images were then exported to ImageJ where TMR was measured.

Blink Measurement

Each recorded 30 second sequence of subject blinking, at each time point, was viewed in a x0.25 slow-motion mode with the VLC Media Player 2.06 (http://www.videolan.org/vlc), and the blink rate per minute analysed at baseline, 0, 1, 5, 10, and 30 minutes after instillation of the different solutions.

The study was conducted in a room with controlled temperature (20 to 23°C) and humidity (44 to 53%). All measurements of the lower tear meniscus radius and the blink-rate were taken on the right eye in primary gaze controlled by a fixation target by a single observer. Analysis of tear meniscus radius was masked against blink-rate count. The examiner was masked to the different drops and time points. To minimise diurnal variation, images were recorded in the morning between 10 and 12 o'clock.

Calculation of Tear Volume Loss and Tear Volume Loss Rate per Blink

Total tear volume was calculated by the equation between TMR and tear volume, which was previously described by Yokoi et al.[29]:

$$\textit{Total Tear Volume } (\mu l) = ((TMR-0.256)/0.038)+6.7$$

The volume loss (TVL) was calculated for both solutions for the time intervals between 0 and 1 minute, 1 and 5 minutes, 5 and 10 minutes, and 10 and 30 minutes after instillation. To calculate the tear volume loss rate per blink in the different time intervals, the tear volume loss was divided by the blink rates that were analysed for the relevant time interval.

8.3. Statistical analyses

Data were tested for normality using the Shapiro-Wilk test. The time course of changes in TMR and blink-rate was statistically analysed using one-way ANOVA on ranks (Kruskal-Wallis test). If significant differences were observed, a Dunnett post-hoc test for multiple comparisons was performed to find time points showing a significant difference to the baseline value. Differences between the test solution effects on TMR and blink-rate at various time points were analysed by the paired-t-test (for normal distribution) and Wilcoxon Signed Ranks Test (for non-normal distribution). Correlations between blink rate and OSDI score were evaluated by Spearman Rank Order Correlation. The data were analysed using SigmaPlot 12 (Systat Software Inc., Chicago, USA).

8.4. Results

8.4.1. Changes in Tear Meniscus Radius

Compared to baseline values (0.33±0.08 mm), TMR with SAL was significantly increased upon application of drop (1.55±0.69 mm) and remained significantly greater at 1 min (0.66±0.36 mm) (ANOVA on ranks with Dunnett post-hoc test; $p<0.05$), but became similar to baseline after 5 mins (0.34±0.08 mm) ($p=0.417$). In

contrast, TMR with SYS (baseline TMR 0.32±0.07 mm) remained significantly increased after application (1.62±0.81 mm), and at 1 min (0.81±0.43 mm) and 5 mins (0.39±0.08 mm) ($p<0.05$) (Figure 8.1). Compared to SAL, TMR with SYS was significantly flatter at 1 min (0.15±0.32 mm; $p=0.044$) and 5 mins (0.05±0.08 mm; $p=0.008$) (Figure 8.2). For all other points in time there was no significant difference between the two solutions.

8.4.2. Changes in Blink Rate

Baseline blink rates per minute with SAL (14.8±7.7) and with SYS (14.9±9.4) were significantly increased upon application of drops (22.5±11.8 and 21.3±11.8) (ANOVA on ranks with Dunnett post-hoc test; $p<0.05$), but became similar to baseline figures after 1 min ($p>0.05$) (Figure 8.3). For all points in time there was no significant difference in blink rate between the two solutions.

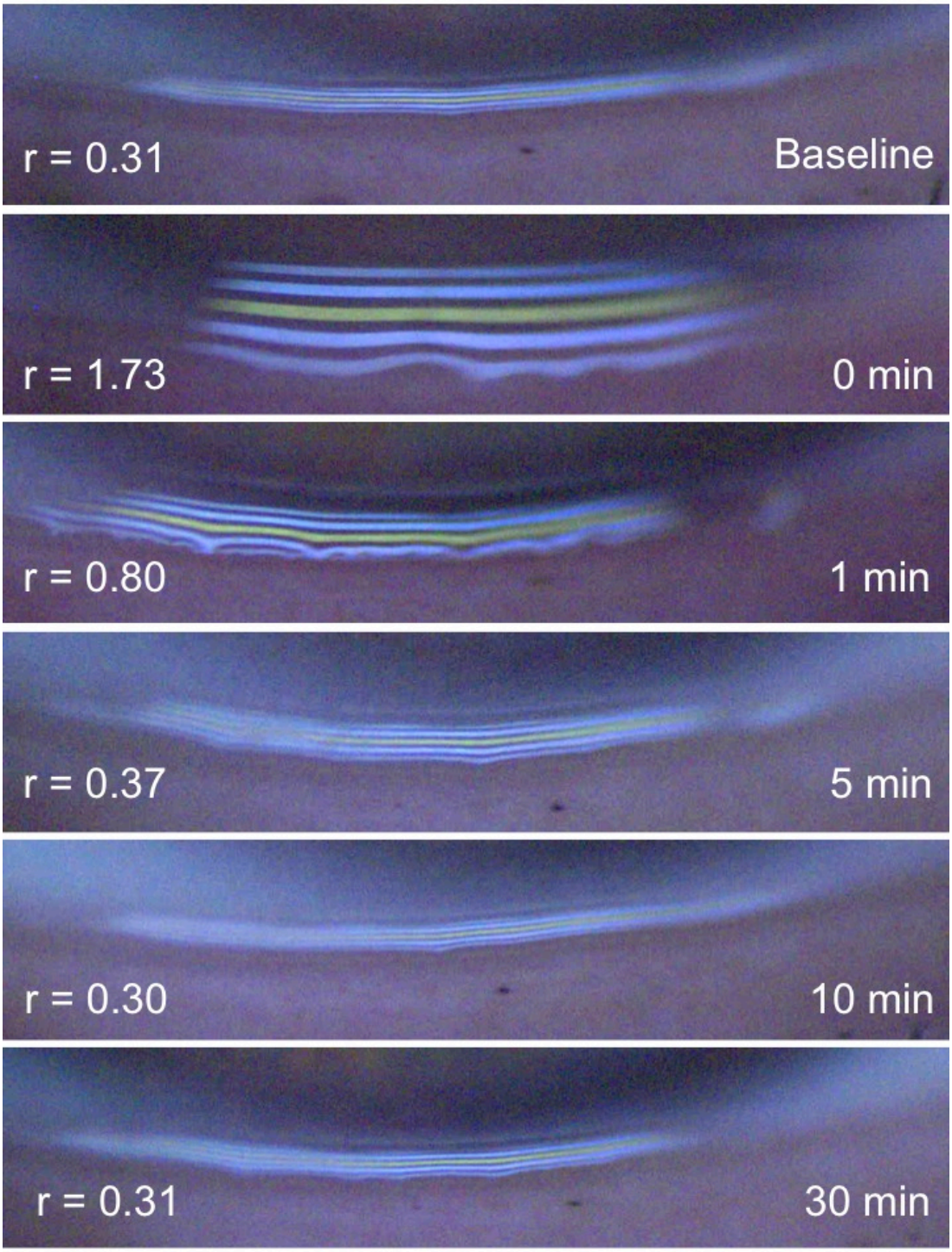


Figure 8.1: Representative PDM images of the dynamic changes in the lower tear meniscus radius before and after instillation of artificial tears containing hydroxypropyl-guar and glycol.

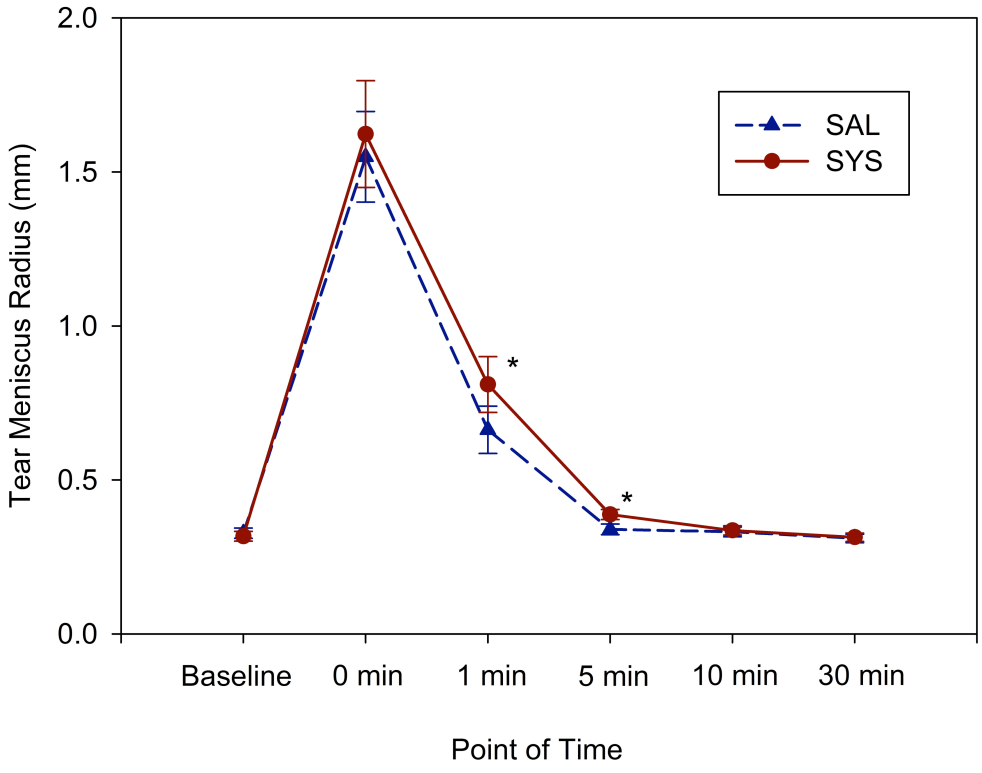


Figure 8.2: Variations in tear meniscus radius after the instillation of artificial tears. Asterisk indicates a statistically significant difference between the two solutions (paired t-test; $p<0.05$). Values are mean ± SE.

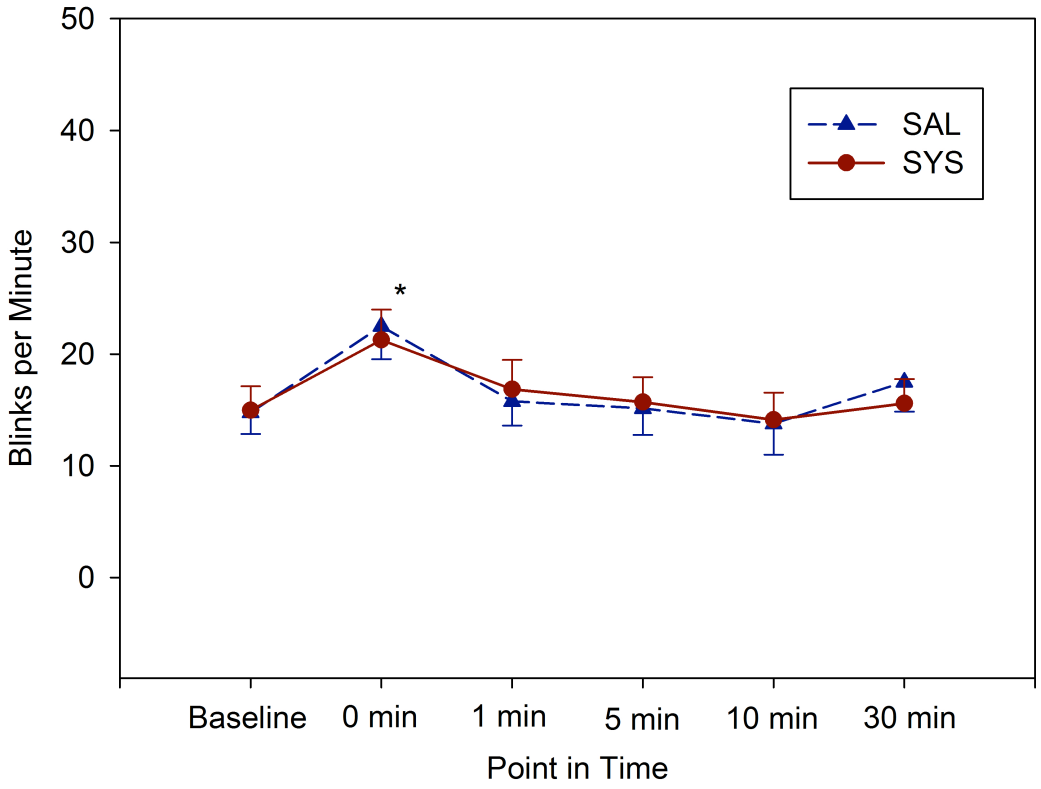


Figure 8.3: Variations in blink rate after the instillation of artificial tears. Asterisk indicates a statistically significant difference to the baseline values (ANOVA on ranks with Dunnett post-hoc test; $p<0.05$). Values are mean ± SE.

8.4.3. Tear Volume Loss and Tear Volume Loss Rate per Blink

The calculated tear volume loss of SAL and SYS in the different time intervals is summarised in Table 8.1.

Time Interval	0 -1 min	1 - 5 min	5 - 10 min	10 - 30 min	Total
SAL (µl)	-23.3 ± 16.5	-11.0 ± 9.0	-0.1 ± 1.0	-0.4 ± 1.5	-34.8 ± 17.8
SYS (µl)	-21.4 ± 16.6	-11.3 ± 10.5	-1.4 ± 1.3*	-0.6 ± 1.5	-34.5 ± 22.3

Table 8.1: Calculated tear volume loss in microliters (mean ± SD) in the different time intervals. Asterisk indicates a statistically significant difference between the two solutions (paired-t-test; $p < 0.001$).

For both solutions there was no statistically significant difference in the calculated rate of tear volume loss per blink when comparing the first time interval 0-1 min (SAL 1.24±1.16; SYS 1.41±1.72 µl/blink) to the second time interval 1-5 mins (SAL 0.68±1.03; SYS 0.83±0.79 µl/blink), and the third time interval 5-10 mins (SAL 0.02±0.11; SYS 0.12±0.12 µl/blink) to the fourth interval 10-30 mins (SAL 0.07±0.17; SYS 0.08±0.23 µl/blink) (ANOVA on ranks with Dunnett post-hoc test; $p<0.05$). The comparison between all other time intervals (first to third and fourth, and second to third and fourth) showed a statistically significant difference in the rate of tear volume loss per blink ($p<0.05$) (Figure 8.4).

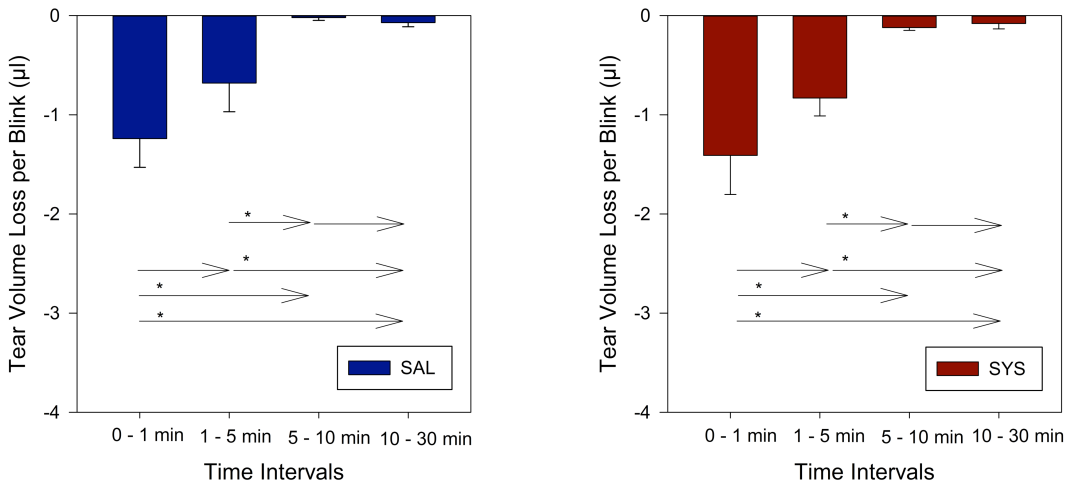


Figure 8.4: Calculated tear volume loss per blink in the different time intervals after the instillation of a 35µl drop. Asterisk indicating a statistically significant difference between the time intervals (ANOVA on ranks with Dunnett post-hoc test; $p<0.05$). Values are mean ± SE.

8.4.4. Correlation between OSDI and Blink Rate

Mean OSDI score at baseline was 10.5±7.7 (SD) with a range from 0 to 27.1. The OSDI score was correlated to the blink rate at baseline (Spearman's rank correlation coefficient, $r=0.550$; $p=0.008$; Figure 8.5). The power calculation of the completed study resulted in a power of >0.77 ($\alpha=0.05$).

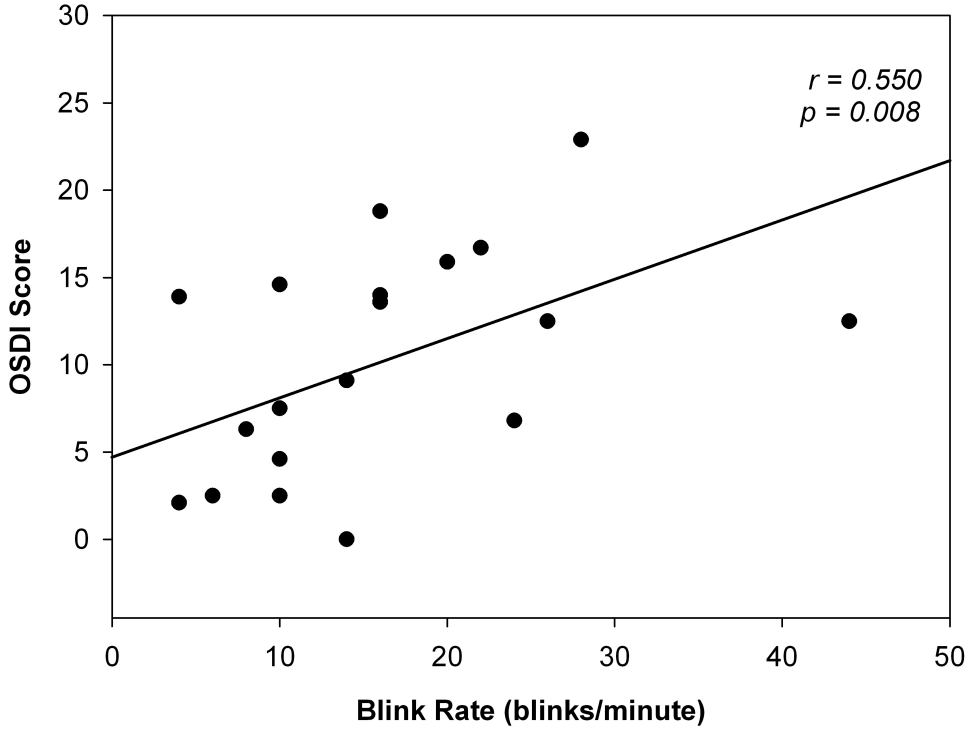


Figure 8.5: Relationship between OSDI score and blink-rate at baseline (Spearman's rank correlation coefficient, r=0.550; p=0.008).

8.5. Discussion

This study reports the use of the custom-made portable digital meniscometer (PDM) to evaluate the dynamic changes of the lower tear meniscus radius after adding artificial tears. Using the PDM, an increase in TMR (and therefore tear volume) was found after instillation, with a return to baseline figures after 5 mins for the saline solution and after 10 mins for the artificial tears containing hydroxypropyl-guar and glycol.

Wang et al.[177, 238] measured the dynamic changes of tear meniscus height (TMH), tear meniscus radius (TMR), and tear meniscus cross-sectional area (TMA) after artificial tear instillation using a custom-made OCT system. They found the tear meniscus parameter returned to baseline 5 mins after instillation of saline (viscosity 1cP), carboxy-methylcellulose sodium (CMC) 0.5% and 1.0% (3 cP and 70 cP), and propylene glycol 0.3% (10 cP). However, they found an increase in tear film thickness and lower tear meniscus variables at instillation with the more viscous drops in healthy patients. Also, using CMC in a concentration of 0.5% and 1.0 % in dry eye patients and controls, Wang et al.[365] used a spectral domain OCT to measure TMH and TMA changes. While in the control group the 0.5% CMC and the 1% CMC persisted for 1 and 15 minutes, in the dry eye group the artificial tears persisted for 5 and 30 minutes. They suggested that the longer retention time is associated with the viscosity of the drop and, furthermore, that in a dry eye patient a lower tear clearance rate might prolong the retention time. In this study, when measuring TMR with the PDM in subjects without significant dry eye, a two times longer retention time was found with the more viscous drop compared to saline. Although in this group the differences between the drops were small but statically significant, a clinically more relevant difference could be expected in dry eye patients, as suggested by Wang et al.[177] Interestingly, the difference of 0.05 mm in TMR after 5 mins, represents a difference in volume of 1.3 µl (Table 1). Estimating a total tear volume of 6.2 µl[62], this represents an increase of about 20%, which might be clinically relevant.

Furthermore, the artificial tears used in this study were specifically formulated to minimise the evaporative loss of tears from the ocular surface, by adding a polar

phospholipid surfactant and mineral oil[370]. Therefore, beside the viscosity, a difference in tear evaporation rate between the two used drops could have impacted the changes in TMR.

Yokoi et al.[29] investigated the relationship between tear volume and TMR measured using a video-meniscometer, concluding that there is a linear relationship between the volume of the instilled saline solution and the measured TMR. Applying the video-meniscometer, they showed that a 0.1 % hyaluronic acid solution resided longer in the tear meniscus than a solution containing 0.1% KCl and 0.4% NaCl[26]. The PDM in this study is based on the video-meniscometer[27], where the tear strip acts as a concave mirror, and likewise changes in tear volume were able to be detected by measuring the dynamics in the TMR.

Besides the volume and the viscosity of the drop, blinking plays an important role in the distribution and drainage of instilled fluid. The lacrimal drainage capacity in young individuals was found to be correlated to the blink rate[364]. Palakuru et al.[164] analysed the blink outcome, defined as the difference in tear volume before and after a blink, upon the instillation of 35µl of 1% CMC. Immediately after the drop was applied, the blink outcome of one blink was increased compared to the blink outcome after five minutes. They concluded that the increase in blink outcome helps to restore balance when the instilled drop overloads the tear system. Zhu and Chauhan[371] used a mathematical model and calculated a drainage rate of 1.174µl per blink for the overloaded tear film. After overloading the tear film by repeatedly instilling saline solution into the tear film for 3 mins, Sahlin et al.[372] reported drainage rates of 1.11 to 4.03µl per blink. In this study the volume loss rates of 1.24

and 1.41µl per blink in the first time interval of 0-1 min are in good agreement with the previously reported values. Interestingly, even though the tear volume after 1 min was significantly diminished, the volume loss rate per blink in the second interval (1 – 5 mins) was not significantly different to that in the first interval. This fact might be explained by the observation of an increase in blink rate upon application of drops with a return to baseline after 1 min. These results favour the interpretation that, during the initial overload phase, the increase in tear volume results in an increase in blink-rate, but that as soon as the volume is reduced to a certain level, a reduction in blink rate keeps the volume loss rate per blink nearly constant. Once the overload is removed, the volume loss rate per blink of the normal tear film stays constant (Figure 5). This mechanism has not previously been reported, although Palakuru et al.[164] argued for a relationship between tear volume and blink rate output based on the analysis of a single blink.

The spontaneous blink rate at baseline in this study compares well with the literature[165, 373]. Upon drop instillation the blink rate increased with no difference in the blink rates between the two solutions. Based on these observations, it is hypothesised that the viscosity of the drop does not influence the effect. However, the difference in viscosities of the two drops used in this study may be too small and the variations in blink rates too large to detect an effect from drop viscosity on blink rate.

Dry eye patients exhibit an increased blink rate in response to the drying of the ocular surface[161, 162, 374]. Although the cohort in this study was very young, we

confirmed a correlation between symptoms evaluated by the OSDI scores and the blink rates.

A limitation of this study may be that completeness of blink was not assessed. Recent studies suggest that not only the frequency but also the completeness of blink may have an effect on dry eye symptoms[165, 166]. Further studies are needed to examine the effect of different types of blinking on the loss of tear film volume.

8.6. Conclusions

In summary, the PDM is able to usefully detect changes in TMR following the instillation of artificial tears. The difference in residence time is likely to reflect the different viscosity and Newtonian properties of these drops. An overload with a large drop may result in an initial increased blink rate. Blink rate at baseline was significantly related to dry eye symptoms.

The published form of this chapter can be found in Appendix 2.4:

Bandlitz S, Purslow C, Murphy PJ, Pult H.

Time course of changes in tear meniscus radius and blink rate

after instillation of artificial tears.

Invest Ophthalmol Vis Sci 2014;55:5842-5847.

CHAPTER 9: Overall Conclusions and Future Work

As stated in Chapter 1, this PhD had the three principal aims to (i) improve the evaluation of the tear meniscus for the clinician by developing an advanced observation device, (ii) investigate the relationship between the tear meniscus parameters TMR and TMH, as well as the effect of area of observation in normal and dry eye patients, and (iii) further explore the impact on the menisci from tear film supplements. From the results of the studies in five experimental chapters 4 to 8 the following conclusions can be made:

1. The newly developed device uses the principal of the reflective video-meniscometer, but can be used on any commercially available digital slit-lamp. This follows the published recommendations of the Dry Eye Workshop that suggests the adaption of reflective meniscometry for general use. A simple iPod touch or an iPhone can be used to project the necessary grid and only an additional holder is necessary to mount the system to the slit-lamp. This new instrument named the Portable Digital Meniscometer (PDM) is a simple, mobile and reasonable device to measure tear meniscus radius, and therefore tear volume, and is suitable for use by clinicians.

2. The new PDM produces accurate and reliable measurements *in vitro* and *in vivo*, and provides similar values for tear meniscus radius, in human studies, to the existing video-meniscometer.

3. PDM and OCT measurements of the TMR are significantly correlated. Since with the PDM no image calibration is needed, it seems to be a quick and non-invasive technique for evaluation of tear fluid quantity. The PDM appears to measure the radius of the central section of the tear meniscus.

4. The PDM is able to non-invasively measure alterations in TMR and TMH along the lower lid. The flatter TMR and higher TMH at the nasal and temporal locations may be caused by the LIPCOF degree of the underlying conjunctiva. To avoid any interference by LIPCOF, it is recommended that TMR and TMH are measured along the lower lid margin below the pupil centre.

5. The PDM is able to usefully detect changes in TMR following the instillation of artificial tears. The difference in residence time is likely to reflect the different viscosity and Newtonian properties of these drops. An overload with a large drop may result in initial increased blink rate. Blink rate at baseline was significantly related to dry eye symptoms.

This PhD thesis documents the development of a new portable and affordable meniscometry device to evaluate tear meniscus height, radii and volume, and has proven that the new device is able to detect changes in tear meniscus in an exact and repeatable manner.

To make the new device available for the clinician and for further laboratory experiments, the next step will be to make the software application available through an applications store were it can be downloaded by the user. However, the image taking and the analysis of the reflective tear meniscus grid in this PhD was performed by the separate software of the digital slit-lamp and by ImageJ. For the future it would be conceivable, to enhance the PDM by allowing the iPod touch camera to take the images of the meniscus and to write an application for subsequent image analysis. Thus, it would offer a tear meniscus radius measurement and therefore tear volume evaluation by just taking one picture with the iPod touch or iPhone. Once this simple, time saving, low-cost and hand-held technology becomes available to the community, this might enable the consumer to use this device at home for self-administered eye tests and measurements. The data collected from these tear film measurements of a broad population in their habitual environment, will provide useful insights and therefore better eye care to patients.

As with the slit-lamp image capture system, the anterior profile of the meniscus on the cross-sectional OCT images is mostly treated as part of a circle with just one radius from the top to the bottom. However, the profile of the meniscus has a more complex shape, as was shown with the OCT measurements in Chapter 6. To investigate this further, the iPod touch or iPhone used as a target in the PDM can be tilted, as described in Chapter 4. This will enable the positioning of the reflection of the white and black bands at different locations on the meniscus profile and may help make analysis of change in the TMR more detailed in the future. Compared to the classic video-meniscometer, where only a few lines can be observed on the meniscus, the new instrument allows the reflection of up to 12 lines at the meniscus,

which means that irregularities in the shape of meniscus are made visible. In combination with the rotatable and moveable PDM, this might enable a detailed color-coded surface topography of the anterior meniscus profile from the bottom to the top along the complete lid margin.

In the literature, the measurement of tear meniscus parameters is mostly performed at the centre of the lower eyelid, directly under the pupil. Some authors reported TMH to be greater at the centre of the lid[185], but others analysed no thinning of the inferior tear meniscus[100], or even reported that the TMH is lower at the centre[25]. At the same time, when calculating TMV the meniscus is assumed to be equal along the lower lid[31, 241], or a correction factor of ¾ is used to account for an unequal distribution[177, 185, 356]. As shown in Chapter 7, tear meniscus regularity is influenced by LIPCOFs. The flatter TMR and higher TMH that were found at the nasal and temporal locations were influenced by the LIPCOF degree of the underlying conjunctiva. It would be useful to investigate whether the measured difference in TMH and TMR also results in an unequal tear volume distribution and whether, as a consequence, a new correction factor for tear volume calculation should be developed for when LIPCOFs are present.

In conclusion, this PhD provides a new reliable and simple device for non-invasive tear meniscus evaluation. Using the PDM, clinicians and researchers in the future will have the opportunity to detect changes in tear fluid volume and therefore to improve the diagnosis and treatment of dry eye patients.

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APPENDICES

Appendix 1: Research Presentations

1.1. A New Portable Digital Meniscometer

Stefan Bandlitz[1,2], Heiko Pult[1,3], Christine Purslow[1], Paul Murphy[1], Anthony J. Bron[4]. [1]School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK; [2]Cologne School of Optometry, Cologne, Germany; [3]Optometry and Vision Research, Weinheim, Germany; [4]Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, UK.

Poster presentation at: Tear Film and Ocular Surface Society, 6th International Conference on the Tear Film and Ocular Surface: Basic Science and Clinical Relevance, Florence, Italy (2009)

Purpose: Reflective meniscometry is a non-invasive method to measure the tear meniscus radius (TMR), useful in dry eye diagnosis. We developed a portable, slit-lamp mounted, digital device (PDM) and compared its accuracy and reproducibility with the standard video-meniscometer (VM), in vitro and in vivo.

Methods: The medians of three consecutive measurements on 5 glass capillaries (radii 0.100 to 0.505 mm) were compared between VM and PDM at two different sessions. Also, the lower tear meniscus radius (TMR) in 20 normal subjects (10M, 10F; mean age 32.3 SD ± 9.3 years) was measured using both techniques. Differences between sessions and instruments were analyzed using Bland-Altman plots, coefficient of repeatability (CR) and paired t-tests.

Results: The PDM and VM were accurate in vitro (95% CI of difference: PDM -0.0134 mm to + 0.0074; p=0.468; VM -0.0282 to + 0.0226; p=0.775), and reproducible between sessions (95% CR: 0.019 and 0.018 respectively). The mean difference between the PDM and VM was 0.0002 (CI – 0.0252 to + 0.0256; p=0.984). In human subjects, there was no significant difference between the mean TMR measured with the PDM (0.34 ± 0.10 mm) and the VM (0.36 ± 0.11) (p=0.124).

Conclusions: This new slit-lamp mounted digital meniscometer appears accurate and reliable, and provided similar values for tear meniscus radius in human studies, to the existing video-meniscometer. The instrument appears suitable for use in both research and clinical practice.

1.2. Comparison of a New Portable Digital Meniscometer and Optical Coherence Tomography in Tear Meniscus Radius Measurement

Poster presentation at: Meeting of the International Society of Eye Research (ISER), Berlin, Germany (2012)

Stefan Bandlitz[1,2], Christine Purslow[1], Paul J Murphy[1], Heiko Pult[1,3]

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Purpose: Non-invasive measurement of tear meniscus radius (TMR) is useful in the assessment of the tear volume and dry eye diagnosis. This study investigates the agreement between a new portable, slit-lamp mounted, digital meniscometer (PDM) and Optical Coherence Tomography (OCT; Zeiss Cirrus HD) in the measurement of the TMR.

Methods: Images of the tear meniscus of 30 normal subjects (8M, 22F; mean age 27.5 SD±9.6 years), recruited from the patient pool of Höhere Fachschule für Augenoptik, Cologne, Germany, were taken using the PDM and the OCT (randomized order). On the PDM and OCT images TMR was measured using ImageJ 1.46b software. In addition, the meniscus on OCT images was sub-divided

vertically into three equal sections and the radius calculated for each sub-section: bottom (BTMR), centre (CTMR) and top (TTMR). The relationship between PDM and OCT measurements was analyzed by Spearman's Rank Coefficient and, differences between PDM and OCT sub-section measurements were evaluated by Bland-Altman plots.

Results: TMR measured with the PDM (0.25±0.06mm) and OCT (0.29±0.09mm) was significantly correlated ($r=0.675$; $p<0.001$). The mean differences between PDM and the sub-sections showed that TMR measured with PDM was flatter (0.07mm; CI 0.05 to 0.10; $p<0.001$) for BTMR, similar (-0.01mm; CI -0.04 to 0.02; $p=0.636$) for CTMR, and steeper (-0.07mm; CI -0.10 to -0.04; $p<0.001$) for TTMR.

Conclusions: PDM and OCT measurements of the TMR are significantly correlated. The PDM appears to measure the radius of the central section of the tear meniscus.

1.3. Evaluation of Lower Tear Meniscus Shape with OCT

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Conference talk at: European Association for Vision and Eye Research (EVER) Annual Congress, Nice, France (2012)

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Purpose: When measuring the tear meniscus radius and calculating tear volume, the anterior radius of the meniscus is assumed to be spherical. This study aimed to define the shape of the meniscus more precisely using high-resolution optical coherence tomography (OCT).

Methods: Images of the lower tear meniscus of 30 normal subjects (8M, 22F; mean age 27.5±9.6yrs), were taken using the Zeiss Cirrus HD OCT. Applying ImageJ software, the tear meniscus height (TMH) was measured and the xy-coordinates of 12 marked points on the anterior tear meniscus curve were determined. With these coordinates a graph was plotted and the best fitting trend-line (defining TM curvature) was calculated. Furthermore, the distance between the edge of the lower eyelid and the vertex of the curve (TMH-V) was calculated and compared to the half TMH (TMH-H).

Results: Mean TMH was 0.24 SD±0.06mm. The mean fitting trend-line appeared to be a quadratic equation (R2 range from 0.908 to 0.996). TMH-V (0.12±0.04mm) and TMH-H (0.12±0.03mm) were significantly correlated ($r=0.62$; $p<0.001$). The 95% LoA showed that the TMH-V could be expected to be up to -0.07 mm below and 0.07 mm above the TMH-H.

Conclusions: With high-resolution OCT the anterior surface of tear meniscus was found to have a parabolic shape, which will help to calculate tear volume more precisely. To know the position of the parabolas vertices is useful when explaining the position of light reflexes from the tear meniscus particularly in reflective meniscometry.

1.4. Tear Meniscus Regularity along the Lower Eyelid

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Conference talk at: Conference of the British Contact Lens Association (BCLA), Manchester, UK (2013)

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Purpose: Non-invasive measurement of tear meniscus radius (TMR) and height (TMH) is useful in the assessment of tear volume and dry eye diagnosis. The tear meniscus is mostly evaluated at the centre of the lower eyelid and, when calculating tear meniscus volume, is either assumed to be equal along the lower lid or a correction factor is used to account for an unequal distribution. This study investigates the capability of a new, portable, slit-lamp mounted, digital meniscometer (PDM) to measure TMR and TMH at different locations along the lower lid.

Methods: Using the PDM, the TMR and TMH of 42 normal subjects (13M, 29F; mean age 27.4 SD±8.2 years) was measured at three locations along the lower lid of

one eye; central (TMR-C; TMH-C), perpendicular below the pupil centre, temporal (TMR-T; TMH-T) and nasal (TMR-N; TMH-N), perpendicular below the limbus. Correlations between the measurements were analysed using the Pearson coefficient and the differences evaluated by Bland-Altman plots and paired t-tests.

Results: Central TMR-C (0.27±0.08mm) and TMH-C (0.20±0.04mm) were significantly correlated to both temporal TMR-T (0.31±0.10mm; r=0.653) and TMH-T (0.27±0.07mm; r=0.561), and nasal TMR-N (0.30±0.11mm; r=0.770) and TMH-N (0.25±0.06mm; r=0.529) ($p<0.001$). TMR-T was 0.041mm flatter ($p=0.002$) and TMH-T mm higher ($p<0.001$), while TMR-N was 0.026mm flatter ($p=0.038$) and TMH-N 0.046mm higher ($p<0.001$) than TMR-C and TMH-C. No significant differences were found between TMR-T and TMR-N ($p=0.159$), or between TMH-T and TMH-N ($p=0.118$).

Conclusions: The PDM is able to non-invasively measure alterations in TMR and TMH along the lower lid. The flatter TMR and higher TMH at the nasal and temporal locations may be caused by variations in tear volume along the lid or by different structure of the underlying conjunctiva in comparison to the central cornea. We therefore recommend measuring TMR and TMH in the central position below the pupil centre.

1.5. Time Course of Changes in Tear Meniscus Radius after Instillation of Artificial Tears

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Purpose: The measurement of tear meniscus radius (TMR) is related to the tear volume. Artificial tears are used to increase tear volume and retention, and to improve tear film quality. This study investigates the capability of a novel slit-lamp mounted, portable digital meniscometer (PDM) to measure alterations in TMR after the instillation of artificial tears.

Methods: Using the PDM, the central TMR of 22 subjects (11M, 11F; mean age 24.3 SD±2.6 years) was measured at baseline and 0, 1, 5, 10, and 30 minutes after instillation of Systane Balance® (SYS) and a saline solution (SAL). A defined drop size of 35µl was applied in one eye in a randomised order with a washout period between the different solutions. The time course of changes in TMR was statistically analysed using repeated measures ANOVA and Dunnett post-hoc test. Differences

between the test solution effects on TMR at various time points were analysed by the paired-t-test or Wilcoxon-Test.

Results: Baseline TMR with SAL (0.33±0.08mm) was significantly increased upon application of drop (1.55±0.69mm) and remained significantly greater at 1min (0.66±0.36mm) ($p<0.05$), but became similar to baseline after 5 mins. In contrast, baseline TMR with SYS (0.32±0.07mm) remained significantly increased on application (1.62±0.81mm), up until 1 min (0.81±0.43mm) and 5mins (0.39±0.08mm) ($p<0.05$). Compared to SAL, TMR with SYS was significantly flatter at 1 min (0.15±0.32mm; $p=0.044$) and 5 mins (0.05±0.08mm; $p=0.008$). For all other points in time there was no significant difference between the two solutions.

Conclusions: The PDM is able to usefully detect changes in TMR following the instillation of artificial tears. The difference in residence time is likely to reflect the different viscosity and Newtonian properties of these drops.

Appendix 2: Papers

2.1. A New Portable Digital Meniscometer

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OPTOMETRY AND VISION SCIENCE

CLINICAL REPORT

A New Portable Digital Meniscometer

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ABSTRACT

Purpose. The aims of this study were (i) to develop a new portable slit-lamp mounted digital meniscometer (PDM) and (ii) to test its accuracy and repeatability compared to the existing Yokoi et al. videomeniscometer (VM).

Methods. We developed a novel application for an iPod or iPhone, which created an illuminated target of parallel black and white bands. This was used as a portable device with which to perform reflective meniscometry. The medians of three consecutive measurements on five glass capillaries (internal radii, 0.100 to 0.505 mm) were compared between VM and PDM at two different sessions. Also, the central lower tear meniscus radius (TMR) in 20 normal subjects (10 males and 10 females; mean [SD] age, 32.3 [9.3] years) was measured using both techniques. Correlations between the instruments were analyzed using the Pearson coefficient. Differences between sessions and instruments were analyzed using Bland-Altman plots, coefficient of repeatability, and paired *t*-tests.

Results. The PDM and VM were accurate in vitro (95% confidence interval [CI] of difference: PDM −0.0134 to +0.0074 mm, p = 0.468; VM −0.0282 to + 0.0226 mm; p = 0.775) and reproducible between sessions (95% coefficient of repeatability, 0.019 and 0.018, respectively). The mean difference between the PDM and VM in vitro was 0.0002 mm (95% CI, −0.0252 to + 0.0256; p = 0.984). In human subjects, mean (SD) TMR measured with the PDM (0.34 [0.10] mm) and VM (0.36 [0.11] mm) was significantly correlated (r = 0.940; p < 0.001), and there was no statistically significant difference between the measured TMR of the instruments (p = 0.124).

Conclusions. This new slit-lamp mounted digital meniscometer produces accurate and reliable measurements and provides similar values for tear meniscus radius, in human studies, to the existing VM. The instrument is suitable for use in both research and clinical practice.

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Key Words: portable digital meniscometer, reflective meniscometry, tear meniscus radius, tear film, dry eye diagnosis

Dry eye is a multifactorial disease resulting in damage to the ocular surface and symptoms of discomfort, principally due to an aqueous deficiency or to increased tear evaporation.[1] The superior and inferior tear menisci together, represent 75 to 90% of the total tear film volume,[2] although a lower estimate, of 27%, has been made.[3] It has been shown that lower tear meniscus curvature (TMR) is directly related to tear volume,[4] which, in turn, is related to tear flow rate.[5] Thus, various tear meniscus parameters, such as radius of curvature and height, which are indicators of the tear film volume, are important in the diagnosis of aqueous-deficient dry eye.[4,6–9] Measurement of tear meniscus height (TMH) has been used in many studies as a surrogate for tear volume and, in clinical practice, is mostly performed with a slit-lamp.[10–15] However, identifying the upper limit of the meniscus at the slit lamp is challenging unless sodium fluorescein is added to the tear film, which in turn renders the test invasive and may introduce errors.

In contrast, the radius of TMR, while more difficult to measure, may be better at predicting tear volume, since it is performed in a noninvasive manner.[7,16–19] Tear meniscus curvature can be evaluated by the use of slit-lamp photography,[6] optical coherence tomography (OCT),[4,18,20–23] or meniscometry.[9,16,17,24–27] Although both OCT and meniscometry measure the tear meniscus radius noninvasively, they have not found wide application among clinicians, either because they are not commercially available in all parts of the world or they are too expensive.[28]

The first photographic meniscometer was introduced by Bron[29] in 1997 and Yokoi et al.[17] in 1999. It consists of a target of 14 black and 13 white lines, each 2 mm wide, attached to a macrocamera.[17]

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A video system with a CCD camera and target consisting of a central white bar of 3.5 mm wide on a black surround was also described. A modification of the video system called the "videomeniscometer," was developed by Yokoi et al.,[9,26] with a target of a series of black metal bars, 4 mm wide and 4 mm apart, set directly in front of the objective lens and illuminated from behind. The meniscus acts as a concave mirror, and the size of the reflected image is used to calculate TMR. However, only three versions of the free-standing videomeniscometer (VM) that was developed from it by Oguz et al.[26] were produced and remain in use.

Another attempt with a prototype of a meniscometer named "dacryomeniscometer" was introduced by Ho et al.[27] While this instrument was originally designed to describe the tear meniscus profile, it was used only for TMH measurements in later studies.[30,31]

Consequently, the aims of this study were (i) to develop a new portable slit-lamp mounted digital meniscometer (PDM) and (ii) to test its accuracy and repeatability compared to the available Yokoi et al. VM.

METHODS

Instrument Development

To project a target onto the anterior curvature of the tear meniscus, an illuminated target was needed. A conventional iPod touch (Apple Inc., Cupertino, CA) with a 3.5-in. multitouch display 7.5 × 5.0 cm (480 × 320 pixel) was used for this purpose. An application software for the iPod touch was developed to generate a grating of parallel black and white bands on the display (Fig. 1). The width of the lines is shown on the display and can be varied between 0.15 and 15.0 mm via the touch screen. Preliminary work indicated that the optimal spacing of the grating was 7.5 mm for visibility and contrast, with a working distance (a) of 50 mm. The working distance was controlled by use of a sliding caliper. In addition, the vertical orientation of the iPod is given in degrees on the display. To define the distance from the tear meniscus, the iPod touch was fixed to a digital photo slit-lamp (BQ900 with IM900 digital imaging module; Haag-Streit, Koeniz, Switzerland). A commercially available iPod touch stand (Xtand, Just Mobile e.K., Berlin, Germany) was modified and mounted on a metal axis on the stand so that it could be fixed to the tonometer post of the slit-lamp (Fig. 2). This setup allowed adjustment of the target in several orientations in relation to the tear meniscus. The target was presented to the tear meniscus with the grating bands disposed horizontally (Fig. 2).

Specular reflection with the slit-lamp was achieved by setting the incidence angle of the target grating equal to the observation angle of the microscope, which was set at 40× magnification.

Imaging of the reflection was achieved using a digital camera (RM 01 CCD camera, 1600 × 1200 pixel; Haag-Streit) incorporated into the slit-lamp and relayed to image-grabbing software (EyeSuite Imaging; Haag-Streit) within a PC. The computer screen had a resolution of 1280 × 1024, producing a total magnification of about 100×, which was the best compromise in terms of resolution and brightness of the image. The images were saved as JPEGs, and a at later point in time, they were opened with ImageJ 1.46 software (http://rsbweb.nih.gov/ij) for analyses. On the image of the reflected grating obtained, the distance between the outer edges of the two black lines (total width of two black lines and one white projected line) was measured using ImageJ (Fig. 3). The central three lines were selected to minimize any impact of an eventually noncircular profile of the meniscus. With a known size of the target (y), distance of the target (a), and the size of the image on the screen (y'), the radius of the tear meniscus can be calculated using the given formula for a concave mirror (Fig. 4).[17]

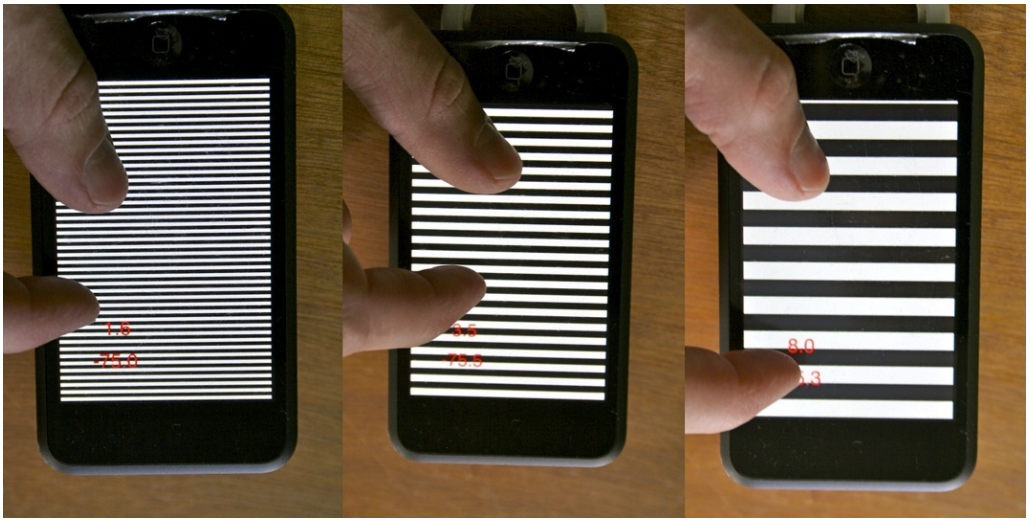

FIGURE 1.
iPod touch (Apple Inc., Cupertino, CA) as a target with adjustable grating width. The numbers on the touch screen give the width of the bars in mm and the vertical orientation of the instrument in degrees.

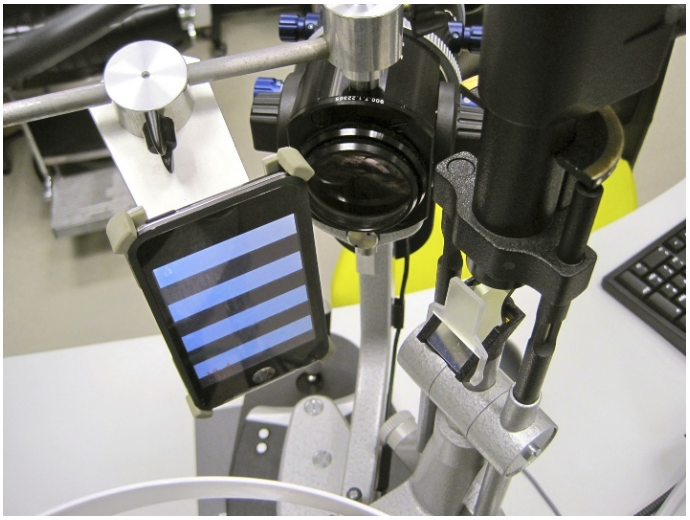

FIGURE 2.
Portable slit-lamp mounted digital meniscometer (BQ900 with IM900 digital imaging module; Haag-Streit).

In Vitro Study

The inner surfaces of five glass capillaries were used as a model of the tear meniscus. The inner diameters and the circularity of the inner surface of the glass capillaries (Hilgenberg GmbH, Malsfeld, Germany) were confirmed by use of a hole-gauge before cutting them lengthwise in half. On the basis of preliminary studies, the medians of three consecutive measurements on the five glass capillaries (radii, 0.100 to 0.505 mm) were compared between the existing VM (Fig. 5) and the new PDM at two different sessions at the same time of day (day 1 and day 2) and after re–set up of the PDM.

In Vivo Study

Twenty subjects (10 males and 10 females; mean age, 32.3 years; range, 23 to 56 years) were randomly selected from the students and staff of the School of Optometry and Vision Sciences at Cardiff University, UK. All procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Human Ethics Committee and were conducted in accordance with the requirements of the Declaration of Helsinki. All subjects gave written informed consent before participating in the study.

Subjects were excluded if they were pregnant or breastfeeding; had a current or previous condition known to affect the ocular surface or tear film; had a history of previous ocular surgery, including refractive surgery, eyelid tattooing, eyelid surgery, or corneal surgery; had any previous ocular trauma; were diabetic; were taking medication known to affect the ocular surface and/or tear film; and/or had worn contact lenses less than 2 weeks before the study. Subjects with a history of dry eye, defined by either an item-weighted McMonnies questionnaire score higher than 14.5 or a fluorescein tear breakup time less than 10 seconds, were excluded.

The lower TMR was measured by one observer using both techniques (VM and PDM) in a randomized order. Care was taken to align both instruments consistently across data collection. The median of three consecutive measurements was recorded for both

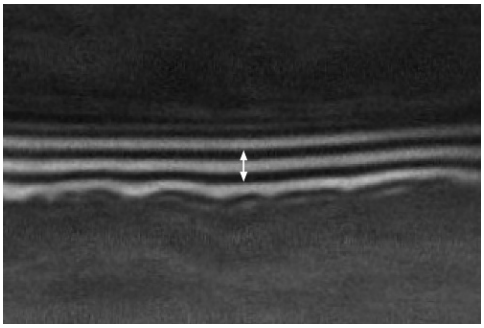

FIGURE 3.
Measurement of line distance on the portable slit-lamp mounted digital meniscometer image using ImageJ 1.46 software.

$$r = \frac{2 \cdot a \cdot y'}{y - y'}$$

r = radius of meniscus curvature
a = target distance
y = target size
y' = image size

FIGURE 4.
Concave mirror formula for calculation of the tear meniscus radius in reflective meniscometry.[17]

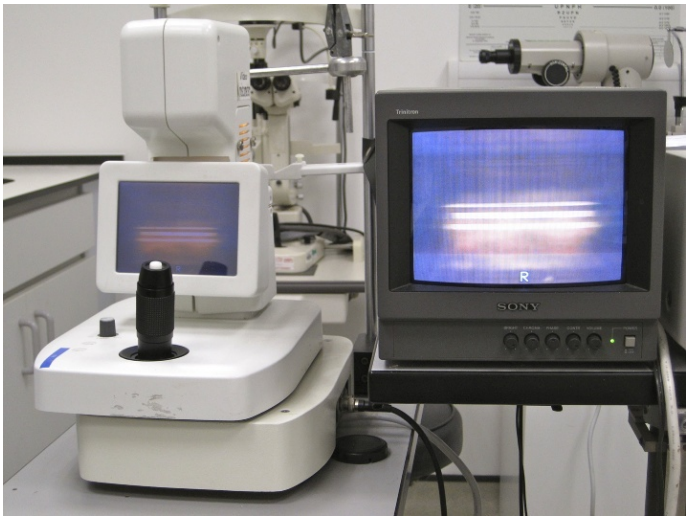

FIGURE 5.
Videomeniscometer.

techniques. On the basis of preexperiments, median instead of mean was chosen. For both techniques, the measurement time was about 2 minutes, with a break of 1 minute between the two instruments. All assessments were of the inferior tear meniscus of the right eye directly below the pupil center with the subject looking straight ahead at a fixed target. The room temperature was 18 to 22°C

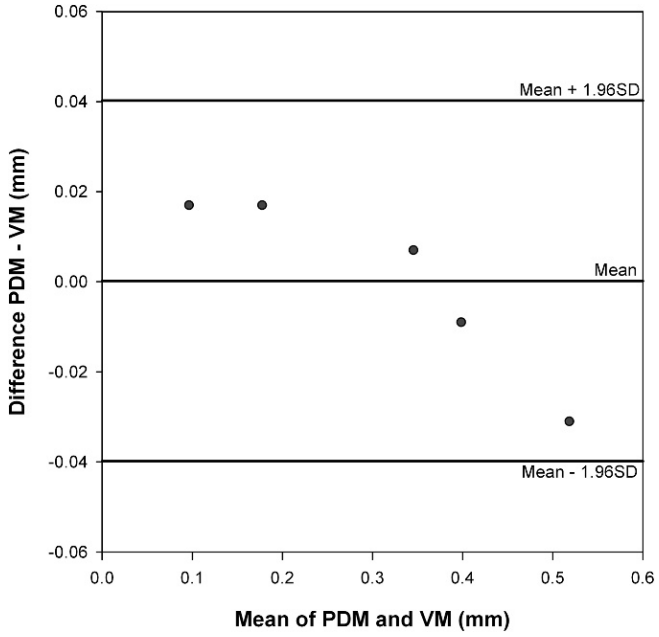

FIGURE 6.
In vitro radius difference between the portable slit-lamp mounted digital meniscometer and videomeniscometer.

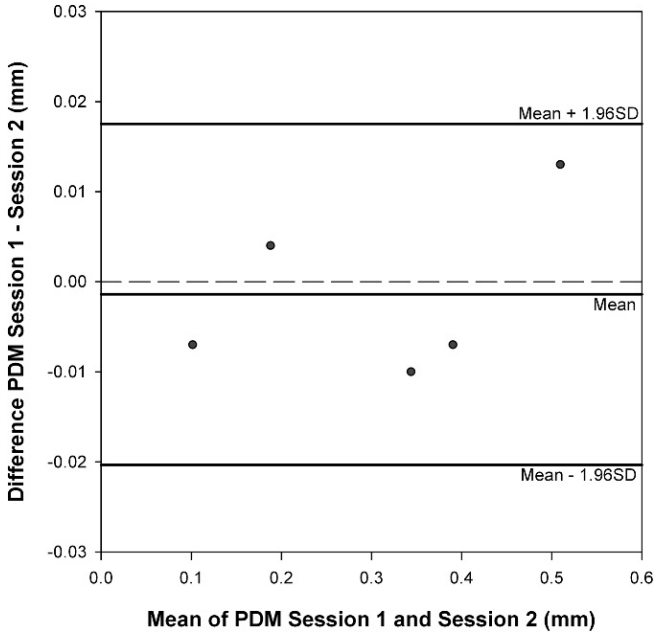

FIGURE 7.
In vitro radius difference between sessions of the portable slit-lamp mounted digital meniscometer.

and the relative humidity was 30 to 40%. To minimize diurnal and interblink variation, measurements were taken in the morning between 10 AM and noon and at 3 to 4 seconds after a blink.

Statistical Analyses

Normal distribution of data was analyzed by Shapiro-Wilk test. Differences between sessions (day 1 and day 2) and instruments were analyzed using Bland-Altman plots, coefficient of repeatability (CR), and paired *t*-tests. The relationship between PDM and VM measurements was analyzed by Pearson product-moment correlation. Data were analyzed using SigmaPlot 12 (Systat Software Inc., Chicago, IL) and BiAS 10 (epsilon-Verlag, Darmstadt, Germany).

RESULTS

In Vitro Study

The measured radii of the five glass capillaries were 0.105, 0.186, 0.349, 0.394, and 0.503 mm for the PDM and 0.088, 0.169, 0.342, 0.403, and 0.534 mm for the VM. The mean difference between the measurements of the two devices was 0.0002 mm (95% confidence interval [CI], −0.0252 to + 0.0256 mm; p = 0.984) (Fig. 6).

Repeated measurements from day 1 and day 2 were not significantly different for the PDM and VM (paired *t*-test: p = 0.468 and p = 0.775, respectively). The 95% CIs around differences indicate acceptable repeatability (95% CI: PDM, −0.0134 to + 0.0074 mm; VM, −0.0282 to +0.0226 mm) and reproducibility between sessions (95% CR: 0.019 and 0.018 mm for PDM and VM, respectively) (Figs. 7 and 8).

In Vivo Study

The mean (SD) TMR of the subjects measured with the PDM was 0.34 (0.10) and 0.36 (0.11) mm of the VM. The PDM measurements were significantly correlated to measures of the VM (Pearson product-moment correlation: r = 0.940, p < 0.001). There was a nonsignificant difference between the measurements taken by the PDM and the VM (mean difference, −0.0151 mm; 95% CI, −0.0285 to −0.0018 mm; paired *t*-test, p = 0.124) in this cohort (Fig. 9).

Examples of a steep (r = 0.19 mm) and a flat tear meniscus radius (r = 0.37 mm) measured with the PDM are shown in Fig. 10.

DISCUSSION

With our newly developed iPod touch–based PDM, we found a good accuracy and reproducibility across the whole range of typical TMR values (Fig. 7). In contrast, the VM seemed to have the tendency to underestimate the TMR for small radii and to overestimate TMR for larger radii (Fig. 8).

This effect was also evident in the comparison between the two methods when the radii measured by the PDM seemed to be more consistent than those measured by the VM (Fig. 6). Since the experimenter was trained in maintaining the alignment of both devices, these apparent differences might be caused by differences in the design and presentation of the targets. While the VM uses

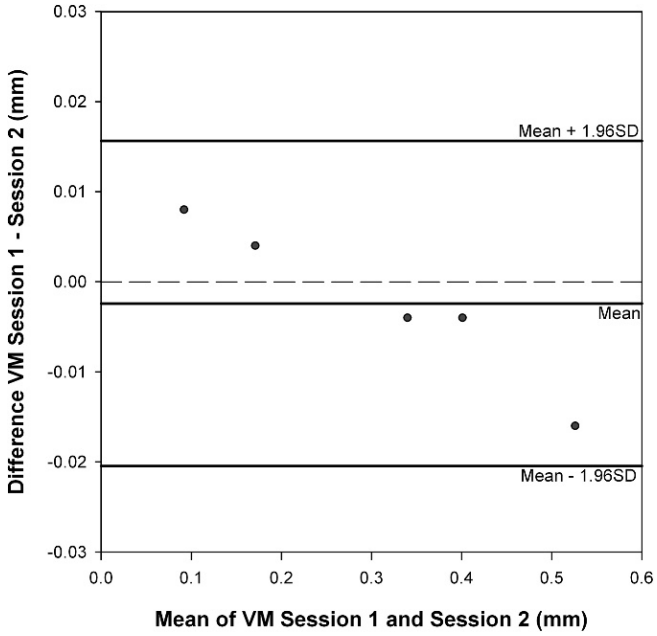

FIGURE 8.
In vitro radius difference between sessions of the videomeniscometer.

metal bars, mounted coaxial with the observation system, the target of the PDM consists of digitally generated bands, which are separated from the observation system. As a result, the PDM target does not interfere with the observation system of the slit-lamp, since the VM target effectively functions as an aperture within the observation system thus influencing the depth of field. A second source of error arises from the working distance of the instrument. While the VM has a working distance of 24 mm, a longer distance of 50 mm is used by the PDM. By looking at the concave mirror formula (Fig. 3), it becomes obvious that the smaller the working distance (a), the greater the error, if the system is not exactly aligned.

In vivo, there was a good agreement between the TMR values of the two instruments. With the PDM, we found a TMR of 0.34 (0.10) mm in a group of patients with normal non–dry eyes. This was not significantly different from the TMR measured with the VM (0.36 ± 0.11 mm) and is in accordance with previously reported measurements using reflective meniscometry in subjects with normal eyes.[9,17] The correlation between the two methods indicates that the PDM provides a valid measurement of TMR. For patients with dry eyes, the reported TMR, measured by reflective meniscometry, has varied between 0.22 (0.09) and 0.25 (0.09) mm,[7,9,26] although some of these reports related to patients with evaporative dry eye.

While meniscometry uses specular reflection to analyze TMR, in OCT, a vertical line scan produces a cross-sectional image of the tear meniscus. On the images taken with an OCT, the 3-point method is used to fit a circle to the anterior border of tear meniscus. The TMR of the lower tear meniscus reported with this method varies from 0.25 (0.05) to 0.46 (0.40) mm for patients with normal eyes and between 0.15 (0.03) to 0.20 (0.08) mm in patients with dry eyes.[18,20,21,23,32]

As in this study, calibration of the original meniscometer system was carried out using glass capillaries.[17] Also using glass capillaries, Kato et al.[33] found no significant differences between TMR measured with the VM and an anterior segment optical coherence tomographer.

For the purpose of calculating meniscus volume, the anterior shape of the meniscus is treated as a part of a circle although it is likely to have a more complex shape.[34] To understand differences in TMR measurements between reflective meniscometry and OCT, it would be helpful to describe the shape of the meniscus more precisely and to analyze the location on the meniscus where the PDM is measuring the meniscus. While OCT and the existing VM have a fixed orthogonal orientation of the target, the PDM allows rotation of the target and therefore a measurement of the meniscus under different angles in the coronal plane. This could be of value in following differences in TMR along the nasal and temporal slopes of the lid. Furthermore, the bandwidth of the target can be easily varied via the touch screen. This enables a finer grating to be projected onto the meniscus, with the possibility of obtaining a more detailed description of the tear meniscus profile.

In the literature, the measurement of tear meniscus parameters is mostly performed at the center of the lower eyelid, directly under the pupil. Some authors report TMH to be greater at the center of the lid,[35] but others find no thinning of the

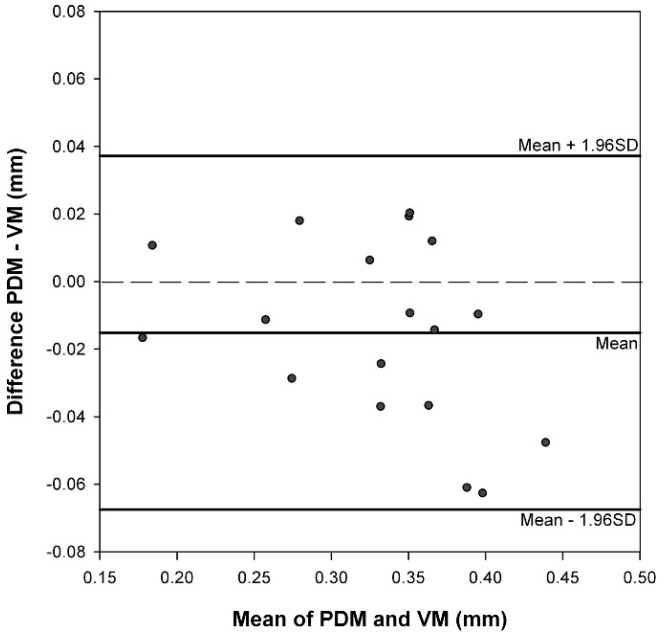

FIGURE 9.
In vivo radius difference between the portable slit-lamp mounted digital meniscometer and videomeniscometer.

inferior tear meniscus,[36] or even that the TMH that is smaller at the center.[13] These differences might be explained by the different techniques used and the different locations at which TMH was measured. At the same time, when calculating tear meniscus volume, the cross section of the meniscus is assumed to be equal along the lower lid,[4,37] or a correction factor of ¾ is used to account for an unequal distribution.[35,38,39] Since the PDM is mounted on a standard slit-lamp, it can be used for measurement of TMH, as well as the TMR at different locations, which will facilitate analysis of tear film distribution along the lid.

CONCLUSIONS

Measuring TMR is a useful noninvasive test for dry eye diagnosis.[9,17–19,23] but existing techniques are either not available commercially or are too expensive for general clinical use. We have developed a PDM that permits accurate and reliable measurements of human tear meniscus radius, can be made generally available, and is suitable for use in both research and clinical practice.

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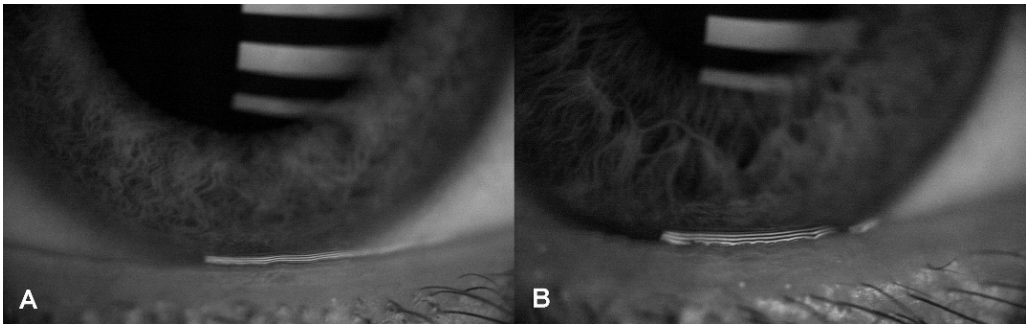

FIGURE 10.
(A) Example of a steep tear meniscus radius (r = 0.19 mm) measured with the portable slit-lamp mounted digital meniscometer. (B) Example of a flat tear meniscus radius (r = 0.37 mm) measured with the portable slit-lamp mounted digital meniscometer.

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2.2. Comparison of a New Portable Digital Meniscometer and Optical Coherence Tomography in Tear Meniscus Radius Measurement

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Comparison of a new portable digital meniscometer and optical coherence tomography in tear meniscus radius measurement

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ABSTRACT.

Purpose: **Non-invasive measurement of tear meniscus radius (TMR) is useful in the assessment of tear volume for dry eye diagnosis. This study investigates the agreement between a new, portable, slit-lamp mounted, digital meniscometer (PDM) and optical coherence tomography (OCT) in the measurement of human TMR.**

Methods: **Images of the tear meniscus at the centre of the lower lid of 30 normal subjects (8M, 22F; mean age 27.5 SD ± 9.6 years) were taken using the PDM and the OCT. On the PDM and OCT images, TMR was measured using IMAGEJ 1.46b software. The meniscus on the OCT images was subdivided vertically into three equal sections and the radius calculated for each: bottom (BTMR), centre (CTMR) and top (TTMR). The relationship between PDM and OCT measurements was analysed using Spearman's rank coefficient, and differences between PDM and OCT subsection measurements were evaluated using Bland–Altman plots.**

Results: **Tear meniscus radius measured with the PDM (0.25 ± 0.06 mm) and OCT (0.29 ± 0.09 mm) was significantly correlated ($r = 0.675$; $p < 0.001$). The mean differences between TMR using the PDM and the subsections from OCT showed that TMR measured with PDM was greater for BTMR (0.07 mm; CI 0.05–0.10; $p < 0.001$), similar for CTMR (−0.01 mm; CI −0.04 to 0.02; $p = 0.636$) and steeper for TTMR (−0.07 mm; CI −0.10 to −0.04; $p < 0.001$).**

Conclusions: **Portable digital meniscometer and OCT measurements of the TMR are significantly correlated, suggesting that the new PDM is a useful surrogate for OCT in this respect. The PDM appears to measure the radius of the central section of the tear meniscus.**

Key words: optical coherence tomography – portable digital meniscometer – reflective meniscometry – tear meniscus radius – tear volume

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Introduction

The tear fluid on the ocular surface is present in the exposed area between the lids, in the conjunctival sac of the upper and lower lids and in the tear menisci along the lid margins. However, the tear menisci hold approximately 75–90% of the overall tear fluid volume and serve as reservoirs, supplying tears to the precorneal tear film (Holly 1985; Savini et al. 2006; Gaffney et al. 2010). The measurement of the anterior curvature radius of the tear meniscus (TMR) is an indicator of tear film volume and has been found to have good dry eye diagnostic accuracies (Mainstone et al. 1996; Bron et al. 1998; Yokoi et al. 1999, 2000; Oguz et al. 2000; Shen et al. 2009). When TMR measurement is carried out in a non-invasive way, this method has great advantages over other invasive tests to evaluate aqueous tear production or volume. These invasive tests, like the Schirmer and Phenol red thread tests, are variably influenced by reflex tearing and show large variations in the test results (Cho & Yap 1993; Tomlinson et al. 2001).

Tear meniscus radius can be measured using a slit-lamp microscope image capture system (Mainstone et al. 1996; Golding et al. 1997; Johnson & Murphy 2006), optical coherence tomography (OCT) (Savini et al. 2006; Palakuru et al. 2007; Wang et al. 2008, 2009; Shen et al. 2009; Li et al. 2012) or reflective meniscometry (Yokoi et al. 1999, 2000, 2005; Oguz et al. 2000; Yokoi & Komuro 2004; Oguz 2008).

With the slit-lamp biomicroscope, the radius of the meniscus can be observed in cross-section. Tear meniscus radius is normally assessed on the captured image by determining the radius of a circle that best fits the curved

anterior meniscal face, with sodium fluorescein instilled in the tear film to improve visibility of the anterior border of the meniscus, although the addition of fluorescein dye will increase tear volume and influence tear meniscus radius (Mainstone et al. 1996; Golding et al. 1997; Creech et al. 1998; Johnson & Murphy 2006). Indeed, the values of TMR obtained from this image capture technique with fluorescein are typically larger than those reported with reflective meniscometry or OCT (Table 1).

In contrast, reflective meniscometry is a non-invasive technique that measures TMR by projecting a target, usually consisting of black and white bands, onto the meniscus at the lower lid margin. The tear meniscus acts as a concave mirror and creates an image of the grating that, when captured by a digital camera, can be analysed using software. Reflex tearing is not stimulated using this technique as a reasonably low level of illumination is sufficient. The original meniscometer was a hand-held device, developed by Yokoi et al. (1999), and later refined by Oguz et al. (2000) into a free-standing version, called the video meniscometer (VM). However, only three versions of the VM currently exist worldwide and the instrument is no longer produced.

Anterior segment OCT of the ocular surface also permits a non-invasive examination of the tear meniscus (Bitton et al. 2007; Wang et al. 2008, 2009; Le et al. 2009). Optical coherence tomography provides cross-sectional high-resolution images of the meniscus and can be applied to the diagnosis and evaluation of dry eye disease (Fercher 2010; Wang et al. 2006; Shen et al. 2008, 2009; Chen et al. 2009; Ibrahim et al. 2010, 2012; Li et al. 2012). Although OCT is useful for tear meniscus measurements, it has not found wide application among clinicians, mainly because it is considered to be too expensive (Savini et al. 2008). On an OCT image, TMR can be measured using the three-point method to fit a circle, thereby producing the TMR (Wang et al. 2008, 2009), although there are some issues in determining the accuracy of these measurements, due to the assumptions made in the instrument image processing algorithms. As with the slit-lamp image capture system, the anterior profile of the meniscus on the cross-sectional OCT images is treated as part of a circle with just one radius from the top to the bottom. However, it is likely that the profile of the meniscus has a more complex shape (Bron et al. 2011). Using slit-lamp image capture to analyse changes in TMR after a blink, Johnson & Murphy (2006) subdivided TMR into two radii: one at the top and one at the bottom of the meniscus. A more detailed description of the radii at the anterior surface of the meniscus has not been addressed in other OCT studies.

Based on the technique of reflective meniscometry for measuring TMR, a new portable, slit-lamp mounted, digital meniscometer (PDM) was recently introduced by the authors (Bandlitz et al. in press). The PDM uses a novel method using an iPod or iPhone screen to produce an illuminated target of parallel black and white bands, which is then projected onto the meniscus at the lower lid margin. The PDM technique has been shown to be accurate and reliable, and is able to provide similar values for TMR to the existing non-portable VM (Bandlitz et al. in press). Because the costs for the PDM are relatively low in comparison with the VM and OCT, it is suggested for use in both research and clinical practice.

While VM and PDM both use reflective meniscometry to measure TMR, OCT uses a different technique. A more detailed description of the shape of the meniscus using a cross-sectional OCT image might therefore help in our understanding of the reflection-based principle of the PDM, specifically the region of the tear meniscus that the PDM image is reflected from. So, the aims of this study were (i) to investigate the agreement between the new PDM and OCT in the measurement of the TMR and (ii) to analyse the location on the tear meniscus from which the PDM image is being reflected.

Material and Methods

Subjects

Thirty healthy subjects (male = 8, female = 22) were randomly selected from the staff and students of the Höhere Fachschule für Augenoptik Köln (Cologne School of Optometry), Cologne, Germany. The mean age was 27.5 years (standard deviation, ±9.3

Table 1. Mean ± standard deviation of central lower tear meniscus radius (TMR) values (mm) of normal subjects reported in the literature using reflective meniscometry, optical coherence tomography and slit-lamp image capture technique.

Reflective meniscometry			Optical coherence tomography			Slit lamp		
Author	Lower TMR	Subjects	Author	Lower TMR	Subjects	Author	Lower TMR	Subjects
Yokoi et al. (1999)	0.37 ± 0.15	45.6 ± 21 years (n = 45)	Wang et al. (2008)	0.46 ± 0.40	45.1 ± 15.4 years (n = 36)	Johnson & Murphy (2006)	0.48	29 (20–53 years) (n = 15)
Maruyama et al. (2004)	0.26 ± 0.09	23.5 ± 5.2 years (n = 11)	Palakuru et al. (2007)	0.39 ± 0.31	32.1 ± 8.7 years (n = 21)	Golding et al. (1997)	0.48 ± 0.21	
Bandlitz et al. (in press)	0.34 ± 0.10	32.3 ± 9.3 years (n = 20)	Wang et al. (2006)	0.26 ± 0.15	40.5 ± 14.1 years (n = 20)	Mainstone et al. (1996)	0.55 ± 0.26	64.4 ± 11.1 years (n = 15)
			Wang et al. (2009)	0.29 ± 0.16	31.7 ± 8.9 years (n = 20)			
			Shen et al. (2009)	0.25 ± 0.05	38.5 ± 12.7 years (n = 47)			
			Li et al. (2012)	0.24 ± 0.05	33.3 (19–56 years) (n = 48)			
Mean ± SD	0.32 ± 0.11			0.32 ± 0.19			0.50 ± 0.24	

years; range, 20–65 years). Subjects were excluded if they were pregnant or breast-feeding; had a current or previous condition known to affect the ocular surface or tear film; had a history of previous ocular surgery, including refractive surgery, eyelid tattooing, eyelid surgery or corneal surgery; had any previous ocular trauma, were diabetic, were taking medication known to affect the ocular surface and/or tear film and/or had worn any types of contact lenses less than 2 weeks prior to the study. Subjects with a history of dry eye, defined by either an item-weighted McMonnies questionnaire score >14.5 or a fluorescein tear break-up time <10 seconds, were excluded. All subjects gave written informed consent before participating in the study. All procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Human Ethics Committee and were conducted in accordance with the requirements of the Declaration of Helsinki.

Instruments

The PDM, based on a conventional iPod touch (Apple Inc., Cupertino, CA, USA) with a 3.5″ multitouch display 7.5 × 5.0 cm (480 × 320 Pixel), was fixed to a digital photo slit-lamp biomicroscope (BQ900 with IM900 digital imaging module; Haag-Streit, Koeniz, Switzerland) (Fig. 1). Imaging of the reflection was captured via a digital camera (RM 01 CCD-camera, 1600 × 1200 pixel; Haag-Streit) incorporated into the slit-lamp biomicroscope and relayed to image-grabbing software (EyeSuite Imaging; Haag-Streit) within a computer. The computer screen had a resolution of 1280 × 1024, producing a total magnification of about 100×, which was the best compromise in terms of resolution and brightness of the image (Bandlitz et al. in press). The iPod touch projects a grating target consisting of a series of white and black bands onto the tear meniscus. With the tear meniscus acting as a concave mirror, the reflected image of the lines was photographed (Fig. 2) and then analysed using ImageJ 1.46 software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2012). The detailed construction of the PDM has been previously described (Bandlitz et al. in press).

The OCT images were obtained using a Cirrus HD-OCT (Carl Zeiss Meditec, Jena, Germany). This instrument uses spectral domain OCT (SD-OCT), with a wavelength of 840 nm to achieve an axial resolution of 5 μm. The cross-sectional images of the tear meniscus in this study were taken using the anterior segment five lines raster method (Fig. 3). In this mode, five parallel vertical lines of 3 mm length and a line distance of 0.25 mm were scanned; each line was composed of 4096 A-scans. As the anterior segment ruler function of the Cirrus HD-OCT is calibrated to measure in a vertical direction within corneal tissue, the images were rotated 90° before measuring the TMR.

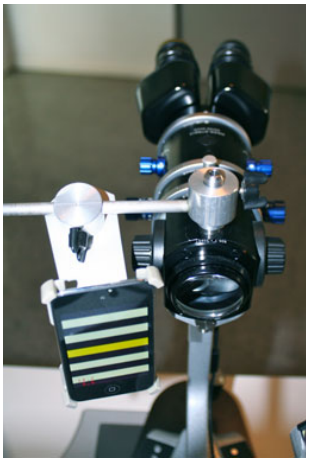

Fig. 1. Portable digital meniscometer (PDM) instrument mounted on a digital imaging slit-lamp (BQ900 with IM900 digital imaging module, Haag-Streit, Koeniz, Switzerland).

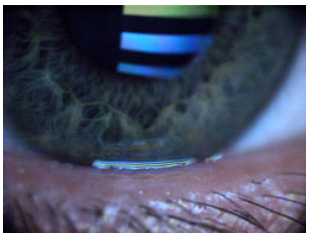

Fig. 2. Reflected image of the Portable digital meniscometer (PDM) lines on the concave central tear meniscus.

Sample calibration

To ensure that on-screen images represented curvature of known dimensions, the inner surface of five glass capillary tubes (radii 0.100–0.505 mm; Hilgenberg GmbH, Malsfeld, Germany) was used as a model for the tear meniscus. The inner diameters of the glass capillaries were confirmed by use of a hole-gauge before cutting them in half. Three OCT scans were taken for each glass capillary. The OCT images were than exported to ImageJ software. Within the ImageJ software, a circle of the confirmed radius of the capillaries was used as a template onto which the OCT image was stretched or compressed until it matched with the circle (Fig. 4). A regression line was then calculated to form a calibration curve, from which all OCT images of the tear meniscus were adjusted before analysis.

Procedures

The study was conducted in a room with controlled temperature (20–23°C) and humidity (44–53%). Portable digital meniscometer and OCT images were taken of the lower tear meniscus of the right eye in primary gaze, directly below the pupil centre in a random order by a single observer. To minimize diurnal and interblink variation, measurements were taken in the morning between 10 and 12 o'clock, and 3–4 seconds after a normal blink. For both techniques, the total measurement time was approximately two minutes, with a break of one minute between the two instruments.

Using ImageJ software, the width of the three bands on the PDM reflected images obtained was measured, and the radius of the meniscus calculated using

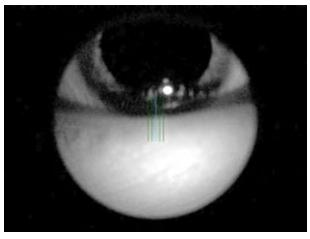

Fig. 3. Tear meniscus cross-sectional imaging with the anterior segment 5 line raster of the Cirrus HD-OCT.

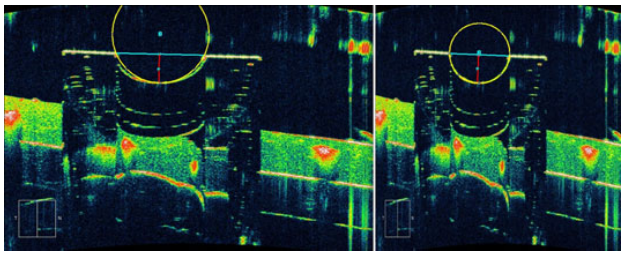

Fig. 4. Optical coherence tomography (OCT) image of a glass capillary before (left) and after (right) image adjustment.

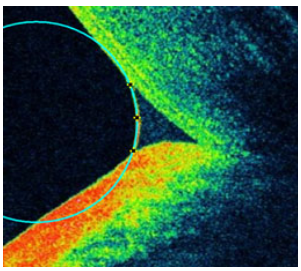

Fig. 5. Tear meniscus radius measured on the optical coherence tomography (OCT) image using the 3-point line-fit technique in ImageJ.

the concave mirror formula. On the OCT images, the three-point circle fit technique was applied to calculate the radius (Fig. 5). In addition, the meniscus on the OCT images was subdivided vertically into three equal sections and the radius calculated for each subsection: top (TTMR), centre (CTMR) and bottom (BTMR) (Fig. 6).

Statistical analyses

Data were tested for normality using the Shapiro–Wilk test and appropriate statistical tests applied. The data were analysed using SigmaPlot 12 (Systat Software Inc., Chicago, IL, USA) and BiAS 10 (epsilon-Verlag, Darmstadt, Germany). The correlation between PDM and OCT measurements was assessed using Spearman's rank coefficient, and differences between PDM and OCT subsection measurements evaluated using paired *t*-testing and Bland–Altman plots.

Results

The mean values and standard deviations plus minimum and maximum values of the lower TMR for each of the different measurements are summarized in Table 2. The radii obtained from the subsections suggest a parabolic curve for the tear meniscus, where the upper portion is flatter and becomes progressively steeper in the central and lower portions.

Tear meniscus radius measured with the PDM (0.25 ± 0.06 mm) and OCT (0.29 ± 0.09 mm) was significantly correlated (Spearman's rank coefficient; $r = 0.675$; $p < 0.001$). The mean differences between PDM and subsections of the OCT images showed that TMR measured with PDM was similar to that measured in the central region by the OCT (−0.01 mm; CI −0.04 to 0.02; paired *t*-test; $p = 0.636$; Fig. 7), but was significantly less for the TTMR (−0.07 mm; CI −0.10 to −0.04; $p < 0.001$; Fig. 8) and significantly increased for the BTMR (0.07 mm; CI 0.05–0.10; $p < 0.001$; Fig. 9).

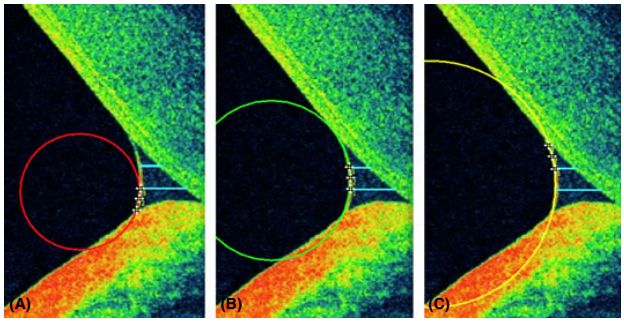

Fig. 6. Best fitted radius for (A) the bottom section of the tear meniscus (BTMR), for (B) the centre section of the tear meniscus (CTMR) and for (C) the top section of the tear meniscus (TTMR).

Discussion

The PDM is an alternative way to non-invasively measure TMR, and this study has demonstrated that it has a high measurement correlation with the existing OCT technique. The PDM therefore has useful potential for TMR measurements that are considered useful in the diagnosis of dry eye, in the determination of tear film distribution and in the evaluation of the effectiveness of dry eye treatments.

Using OCT or reflective meniscometry, average TMR values of the lower central meniscus of normal subjects in previous studies have been reported to range from 0.24 ± 0.05 mm to 0.46 ± 0.40 mm (Table 1). The results from this study are within this range. This is important, because non-invasive measurement of lower TMR has showed good diagnostic accuracy (92% sensitivity and 87% specificity; cut-off value 0.18 mm) in the diagnosis of aqueous-deficient dry eye (Shen et al. 2009). In contrast, the average TMR found using the invasive, slit-lamp fluorescein technique ranges from 0.48 ± 0.21 mm to 0.55 ± 0.26 mm (Table 1), which gives with a cut-off value of 0.35 mm (80% sensitivity and 87% specificity), as suggested by Mainstone et al. (1996).

Kato et al. (2010) reported a significant linear correlation between TMR values measured with VM (0.34 ± 0.21 mm) and OCT (0.35 ± 0.26 mm) in a mixed group consisting of 14 normals, 25 dry eye and 14 epiphora subjects. In their study, they used the RTVue-100 OCT (Optovue Inc.,

Table 2. Mean ± standard deviation and minimum and maximum values (mm) of the lower tear meniscus radius (TMR) for each of the different measurements of central lower TMR.

	Mean ± SD (mm)	Minimum (mm)	Maximum (mm)
TMR-PDM	0.25 ± 0.06	0.11	0.36
TMR-OCT	0.29 ± 0.09	0.18	0.56
TTMR-OCT	0.32 ± 0.10	0.16	0.56
CTMR-OCT	0.26 ± 0.09	0.11	0.49
BTMR-OCT	0.18 ± 0.07	0.09	0.39

TMR-PDM= tear meniscus radius measured with PDM; TMR-OCT= tear meniscus radius measured wit OCT; TTMR-OCT= tear meniscus radius measured with OCT in the top-section of the meniscus; CTMR-OCT= tear meniscus radius measured with OCT in the centre-section of the meniscus; BTMR-OCT= tear meniscus radius measured with OCT in the bottom-section of the meniscus.

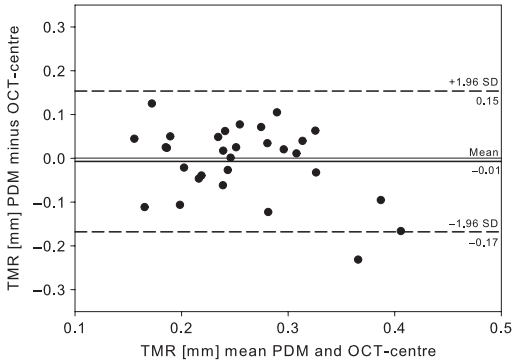

Fig. 7. Differences between Portable digital meniscometer (PDM) and optical coherence tomography (OCT) in the centre section.

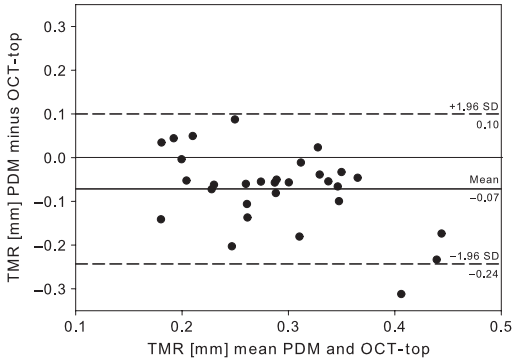

Fig. 8. Differences between Portable digital meniscometer (PDM) and optical coherence tomography (OCT) in the top section.

Fremont, CA, USA), which is also a SD-OCT with an axial resolution of 5 μm, similar to the OCT used in this study. With the Cirrus HD-OCT, we were able to measure TMR by the help of an external image analysing software.

However, there is a significant problem with using the OCT to describe the TMR shape. The dimensions of the images produced by an OCT suffer from distortions in the image paths that cannot be assessed easily. One of these distortions is the 'fan distortion'. It is conditioned by the design of the scanner and the arrangement and design of the mirror and the collimator lens (Ortiz et al. 2011; Siedlecki et al. 2012), but it has the effect that a flat surface appears to be bent. Further distortions, called 'optical distortions', are caused by variations in the refractive indices of the tissue that is being measured (Ortiz et al. 2011; Siedlecki et al. 2012). The higher the refractive index of the tissue, the longer the light takes to go through the tissue: this has the result that a measuring scale calibrated to measure corneal thickness, for instance, cannot be used to measure other tissue structures. To perform reliable measurements with the OCT despite the resulting distortions, specialist algorithms are required to eliminate these errors (Westphal et al. 2002; Dunne et al. 2007). However, such algorithms are part of the OCT software and are not disclosed to the users of the instrument. In this study, the Cirrus HD-OCT (Carl Zeiss Meditec) was used. Within its anterior eye module, the ruler measures only vertical distances, with the scale factor calibrated for measuring corneal tissue only. Because the tear meniscus images are produced in air, the 'in tissue' algorithm corrections were no longer appropriate, and so all OCT images were analysed within separate software programmes. To calibrate the distances and curvatures on the images, OCT images of glass capillaries with known radii were used and then stretched or compressed until no distortions were observed for the first interface. In contrast, there was no need to equalize the PDM images, which made the analysing process easier. The PDM digital images can be directly used and, with the known pixel/mm ratio, distances in all directions can be measured without any transformation.

Our study showed that the PDM measures the radius of the central section of the tear meniscus. To our knowledge, this is the first OCT study in which the meniscus was subdivided into three different sections for detailed analyses. As might be expected from a casual perusal of the tear meniscus cross-sections, the steepest TMR was found in the bottom third and the flattest TMR in the top third of the

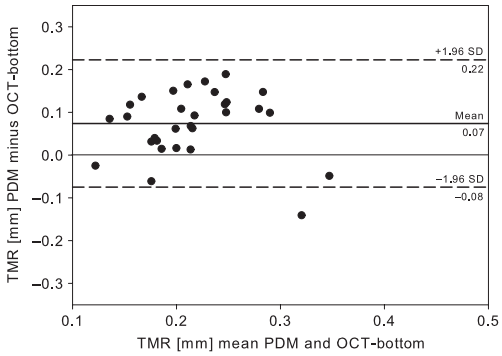

Fig. 9. Differences between Portable digital meniscometer (PDM) and optical coherence tomography (OCT) in the bottom section.

meniscus. In a study by Johnson & Murphy (2006), where they used the slit-lamp image capture technique to measure changes in TM after a blink, the TMR was calculated at the top (TMR_t) and at the bottom (TMR_b) of the meniscus. On eye opening, they found (TMR_t) and (TMR_b) to be similar, indicating an approximately circular meniscus profile, while only 1-second later the radius of the top section was 0.19 mm flatter than that of the bottom section. Thereafter, this difference in radii stabilized.

In this study, the measurements were completed 3–4 seconds after a blink and the TMR of the top third was found to be 0.14 mm flatter than that of the bottom third of the meniscus. Although a non-invasive technique was used and three subsections instead of two, their findings of a flatter TMR at the top of the meniscus were confirmed by this study.

During the first 1.5 second following the blink, Johnson & Murphy (2006) suggested that TMR increases by about 20%, while others observed the lower TMR to be stable during the interblink period (Yokoi et al. 1999; Johnson & Murphy 2006; Palakuru et al. 2007). This discrepancy is most likely the result of the different techniques used or might be due to the observation that only some parts of the meniscus change, while other parts stay stable following a blink (Johnson & Murphy 2006). To investigate this further, the iPod touch or iPhone used as a target in the PDM can be tilted. This will enable the positioning of the reflection of the white and black bands at different locations on the meniscus profile and may help make analysis of changes in TMR more detailed in the future.

Conclusions

Portable digital meniscometer and OCT measurements of the TMR are significantly correlated. Because with the PDM no image calibration is needed, it seems to be a quick and non-invasive technique for evaluation of tear fluid quantity. The PDM appears to measure the radius of the central section of the tear meniscus.

Acknowledgments

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Conflict of Interest

None.

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2.3. The Relationship between Tear Meniscus Regularity and Conjunctival Folds

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FEATURE ARTICLE ON LINE

The Relationship between Tear Meniscus Regularity and Conjunctival Folds

Stefan Bandlitz*, Christine Purslow†, Paul J. Murphy‡, and Heiko Pult§

ABSTRACT

Purpose. To investigate the capability of a new portable digital meniscometer (PDM) to measure tear meniscus radius (TMR) and tear meniscus height (TMH) at different locations along the lower lid and to evaluate relationships between tear meniscus regularity and the degree of lid-parallel conjunctival folds (LIPCOFs).

Methods. Using the PDM, the TMR and TMH of 42 subjects were measured at three locations along the lower lid of one eye: central, perpendicularly below the pupil center (TMR-C, TMH-C), and temporal (TMR-T, TMH-T) and nasal (TMR-N, TMH-N), perpendicularly below the limbus. Nasal and temporal LIPCOF grades were recorded. Correlations between the measurements were analyzed using the Pearson coefficient (or Spearman rank in nonparametric data), and the differences were evaluated by paired *t* tests or analysis of variance and *post hoc* Fisher least significant difference test.

Results. Temporal TMR was 0.041 mm flatter ($p = 0.002$) and TMH-T was 0.063 mm higher ($p < 0.001$), whereas TMR-N was 0.026 mm flatter ($p = 0.038$) and TMH-N was 0.046 mm higher ($p < 0.001$) than TMR-C and TMH-C. Temporal LIPCOF grades were significantly correlated to temporal alterations in TMH ($r = 0.590$; $p < 0.001$) and TMR ($r = 0.530$; $p < 0.001$), and nasal LIPCOF grades were significantly correlated to nasal alterations in TMH ($r = 0.492$; $p = 0.001$) and TMR ($r = 0.350$; $p = 0.023$).

Conclusions. The PDM is able to noninvasively detect significant differences in TMR and TMH along the lower lid. The flatter TMR and higher TMH at the nasal and temporal locations are associated with increased LIPCOF. Because increased LIPCOF scores may affect tear film disruption along the lower lid, measuring TMR and TMH at the central position below the pupil may provide the best intersubject reliability.

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Key Words: tear meniscus regularity, lid-parallel conjunctival folds, LIPCOF, portable digital meniscometer, tear volume, reflective meniscometry

In the diagnosis of dry eye, an evaluation of tear meniscus is an important parameter. The tear menisci hold about 75 to 90% of the overall tear fluid volume and a tear meniscus reduction correlates to a decreased tear volume.[1–6] The measurements of tear meniscus height (TMH) and tear meniscus radius (TMR) and the calculation of the cross-sectional area are limited to one dimension or, in the case of the area, to two dimensions. Because the meniscus is spread along the eyelid margins, the length of the lid is used to calculate the tear meniscus volume. As the eyelids are curved, the eyelid length measured on an image is adjusted by a multiplication factor of 1.294, according to Tiffany et al.[7]

In the published literature, the measurement of tear meniscus parameters is mostly performed at the center of the lower eyelid, directly under the pupil. Some authors report TMH to be greater at the center of the lid,[8] but others find no thinning of the inferior tear meniscus,[9] or even that the TMH is smaller at the center.[10] These differences might be explained by the different techniques used, the timing of such measures after a blink, and the different areas of observation. At the same time, when calculating tear meniscus volume, the meniscus is assumed to be equal along the lower lid,[7,11] or a correction factor of 3/4 is used to account for an unequal distribution.[8,12,13]

Lid-parallel conjunctival folds (LIPCOFs) are folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin. Lid-parallel conjunctival folds were described as a subtype

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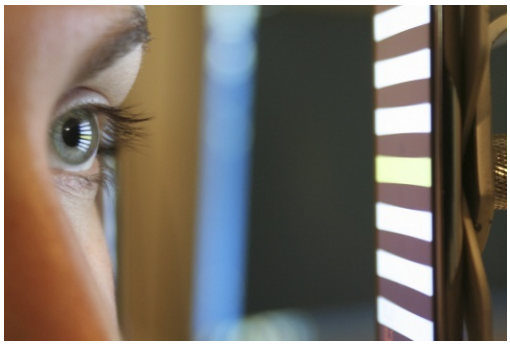

FIGURE 1.

Patient positioned in front of the slit lamp–mounted PDM. The grid on the screen of the iPod touch is reflected by the cornea and the lower tear meniscus. A color version of this figure is available online at www.optvissci.com.

that might represent a mild stage of conjunctivochalasis.[14] Like conjunctivochalasis, LIPCOFs are located in the tear meniscus area and both are assumed to interfere with the meniscus.[15–18]

Recently, an iPod touch–based system, named the portable digital meniscometer (PDM), has been developed to measure TMR. It has been demonstrated as giving accurate and reliable measurements at the central position, which were significantly correlated to optical coherence tomography (OCT) and video-meniscometer values.[19,20] It is not known how effective this new system is at assessing TMH and TMR at different locations along the lid margin.

The aims of this study are (1) to investigate the capability of the new slit lamp–mounted PDM to measure TMH and, for the first time, TMR at different locations along the lower lid and (2) to evaluate any relationships between tear meniscus regularity and the degree of LIPCOF.

METHODS

Subjects

Forty-two subjects (male, 13; female, 29) were randomly selected from the staff and students of the Höhere Fachschule für Augenoptik Köln (Cologne School of Optometry), Cologne, Germany. The mean (±SD) age of the subjects was 27.4 (±8.2) years (range, 20 to 67 years). Subjects were excluded if they were pregnant or breast-feeding; had a current or previous condition known to affect the ocular surface or tear film; had a history of previous ocular surgery, including refractive surgery, eyelid tattooing, eyelid surgery, or corneal surgery; had any previous ocular trauma; were diabetic; and were taking medication known to affect the ocular surface and/or tear film. Because contact lens wear was shown to influence the tear meniscus and LIPCOF grade,[15] all subjects were not allowed to wear contact lenses during and 2 weeks before the study.

Each subject's symptoms were evaluated using the Ocular Surface Disease Index (OSDI) questionnaire and afterward the total OSDI scores were calculated.[21] The subjects were then classified into symptomatic (OSDI score $\geq$ 13) and asymptomatic (OSDI score $<$ 13) patients.[22]

All subjects gave written informed consent before participating in the study. All procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Human Ethics Committee and were conducted in accordance with the requirements of the Declaration of Helsinki.

Instrumentation and Procedures

A newly developed slit lamp–mounted PDM was used to measure TMH and TMR along the lower eyelid. The PDM is based on an application that creates a grid of black and white gratings on the screen of an iPod touch or an iPhone (Apple Inc,

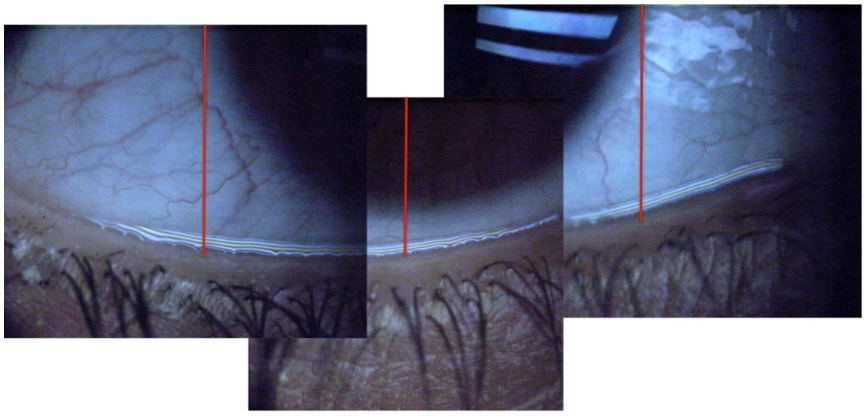

FIGURE 2.

Reflected image of the PDM lines on the concave temporal, central, and nasal tear meniscus. The picture is a composition of the three single slit lamp images with the red line marking the measuring location. The greater the line distance at the location, the flatter the TMR. A color version of this figure is available online at www.optvissci.com.

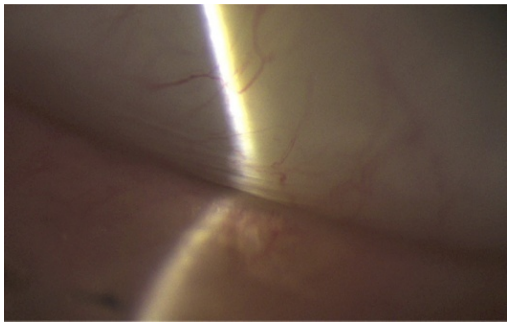

FIGURE 3.
Real slit lamp image of LIPCOF grade 3 at the temporal position. A color version of this figure is available online at www.optvissci.com.

Cupertino, CA) (Fig. 1). The tear meniscus acts as a concave mirror and creates an image of the grating that, when captured by a digital slit lamp camera (BQ900 with IM900 digital imaging module, Haag-Streit, Koeniz, Switzerland), can be analyzed using ImageJ 1.46 software (http://rsbweb.nih.gov/ij). The detailed construction of the PDM has been previously described.[19,20] Specular reflection with the slit lamp was achieved by setting the incidence angle of the target grating equal to the observation angle of the microscope, which was set at 40× magnification. The PDM is mounted on a metal axis and fixed to the tonometer post of the slit lamp and therefore the target can be rotated to avoid shadowing caused by the nose. Using the PDM, TMH and TMR were measured in a randomized order at three locations along the lower lid of one eye: central, perpendicularly below the pupil center (TMR-C, TMH-C), and temporal (TMR-T, TMH-T) and nasal (TMR-N, TMH-N), perpendicularly below the limbus (Fig. 2). An earlier study showed that the PDM measures the radius of the central section of the tear meniscus.[20] The anterior surface of the tear meniscus was found to have a parabolic shape,[23] and PDM measurement of the central section of the tear meniscus was found to be in good agreement with the OCT three-point line-fit technique, where the bottom, center, and upper boundaries of the anterior meniscus surface were delineated.[20]

To minimize diurnal and interblink variation, images were recorded in the morning between 10 and 12 o'clock and 3 to 4 seconds after a normal blink.

Lid-parallel conjunctival folds were evaluated without fluorescein with a slit lamp microscope (BQ900, Haag-Streit) using 25× magnification (Fig. 3). The LIPCOF evaluation was performed

TABLE 1.
Optimized grading scale of LIPCOF[24,25]

LIPCOF grade	
0	No conjunctival folds
1	One permanent and clear parallel fold
2	Two permanent and clear parallel folds (normally <0.2 mm)
3	More than two permanent and clear parallel folds (normally >0.2 mm)

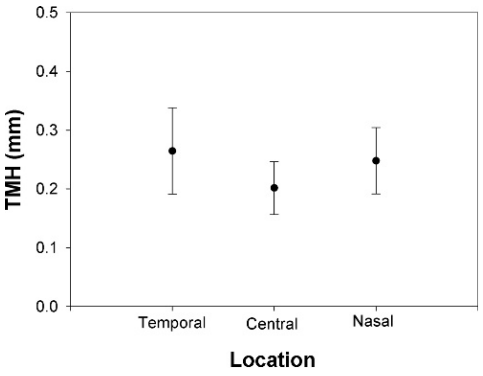

FIGURE 4.
Tear meniscus height at the temporal, central, and nasal position of the lower eye lid.

in the area perpendicular to the temporal and nasal limbus on the bulbar conjunctiva above the lower lid, at the same location where TMH and TMR were measured. Lid-parallel conjunctival fold grade was classified using the optimized grading scale (Table 1).[24,25] Care was taken to differentiate LIPCOFs from microfolds, which are less well organized and around three times smaller than LIPCOFs.[26] To avoid any influence of blinking on the presentation of LIPCOFs, the folds were also classified 3 to 4 seconds after a normal blink.

The study was conducted in a room with controlled temperature (20 to 23°C) and humidity (44 to 53%). All lower tear meniscus measurements and LIPCOF evaluations were taken on the right eye in primary gaze in a randomized order by a single observer. Analysis of tear meniscus parameters was masked against LIPCOF grading.

Statistical Methods

Data were tested for normality using the Shapiro-Wilk test and appropriate statistical tests were applied. Correlations were calculated with Pearson correlation (or Spearman rank in nonparametric

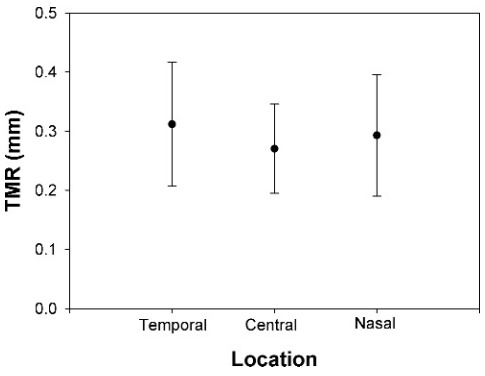

FIGURE 5.
Tear meniscus radius at the temporal, central, and nasal position of the lower eye lid.

TABLE 2.
Correlation of LIPCOF grades with tear meniscus regularity

	Temporal LIPCOF grades		Nasal LIPCOF grades	
	Spearman correlation coefficient	p	Spearman correlation coefficient	p
Difference between temporal and central TMH, mm	0.590	<0.001		
Difference between temporal and central TMR, mm	0.530	<0.001		
Difference between nasal and central TMH, mm			0.492	0.001
Difference between nasal and central TMR, mm			0.350	0.023

data). The differences between the locations along the lower lid were calculated with a paired *t* test. To detect the differences among the LIPCOF groups, one-way analysis of variance and *post hoc* Fisher least significant difference tests were used ($p < 0.05$). The data were analyzed using SigmaPlot 12 (Systat Software Inc, Chicago, IL).

RESULTS

Regularity of TMH

Central TMH (0.20 ± 0.04 mm) was significantly correlated to TMH-T (0.27 ± 0.07 mm; $r = 0.561$, $p < 0.001$) and TMH-N (0.25 ± 0.06 mm; $r = 0.529$, $p < 0.001$). Temporal TMH (0.063 ± 0.061 mm, $p < 0.001$) and TMH-N (0.046 ± 0.044 mm, $p < 0.001$) were both significantly higher than TMH-C (Fig. 4). However, no significant differences were found between TMH-T and TMH-N ($p = 0.118$).

Regularity of TMR

Central TMR (0.27 ± 0.08 mm) was significantly correlated to TMR-T (0.31 ± 0.10 mm; $r = 0.653$) and TMR-N (0.30 ± 0.11 mm; $r = 0.770$) ($p < 0.001$). Temporal TMR (0.041 ± 0.082 mm, $p = 0.002$) and TMR-N (0.026 ± 0.076 mm, $p = 0.038$) were both significantly flatter than TMR-C (Fig. 5). No significant differences were found between TMR-T and TMR-N ($p = 0.159$).

Relationship between LIPCOF Grades and Tear Meniscus Regularity

Temporal LIPCOF scores (1.43 ± 0.86) were significantly correlated to nasal LIPCOF scores (0.57 ± 0.79) ($r = 0.317$; $p < 0.05$). Temporal LIPCOF scores were significantly correlated to the difference between TMH-T and TMH-C ($r = 0.590$; $p < 0.001$) and to the difference between TMR-T and TMR-C ($r = 0.530$; $p < 0.001$), whereas nasal LIPCOF scores were significantly correlated to the difference between TMH-N and TMH-C ($r = 0.492$; $p = 0.001$) and to the difference between TMR-N and TMR-C ($r = 0.350$; $p = 0.023$) (Table 2).

However, with temporal LIPCOF grades of less than or equal to 1, the temporal TMH and TMR were similar to the central TMH and TMR, whereas for LIPCOF grades greater than or equal to 2, they were significantly different (Figs. 6 and 7). Similarly, for the nasal LIPCOF grades of less than or equal to 1, the nasal TMH and TMR were not different from the central TMH and TMR but were significantly different for LIPCOF grades of 2 compared with grade 0 (Figs. 8 and 9).

Dry Eye Symptoms and LIPCOF Grades

Mean (±SD) OSDI score was 10.7 (±7.3) with a range from 0 to 32.5. The OSDI scores and LIPCOF grades for the asymptomatic and symptomatic subjects are summarized in Table 3. There was a statistically significant difference ($p = 0.039$) in temporal LIPCOF

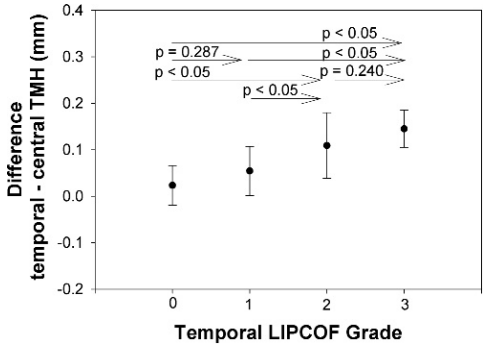

FIGURE 6.
Mean difference between the temporal and central TMH in the four subgroups with different LIPCOF grades.

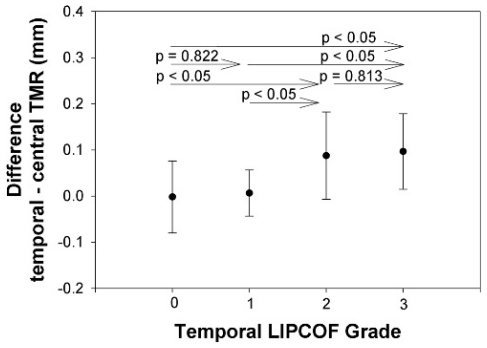

FIGURE 7.
Mean difference between the temporal and central TMR in the four subgroups with different LIPCOF grades.

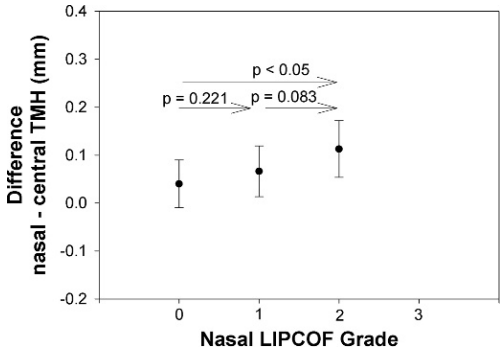

FIGURE 8.
Mean difference between the nasal and central TMH in the four subgroups with different LIPCOF grades.

grades between the asymptomatic and symptomatic subjects, whereas there was no statistical difference (p = 0.964) for the nasal LIPCOF grades.

DISCUSSION

This study has found that the PDM was able to detect variations of TMH and TMR at different locations along the lower lid. The results for the central TMH and TMR were within the range of previous values reported for central TMH (0.10 ± 0.04 mm to 0.46 ± 0.17 mm) and central TMR (0.15 ± 0.03 mm to 0.55 ± 0.26 mm).[27–32]

Temporal and nasal TMH were significantly higher than central TMH. This is in agreement with the observation of Garcia-Resua et al.,[10] although they reported slightly lower values. However, they measured TMH as the distance between the darker edge of the lower eyelid and the upper limit of the brightest reflex of the meniscus, whereas in this study, the upper limit of the tear meniscus was measured. However, identifying the upper limit of the meniscus at the slit lamp is challenging unless sodium

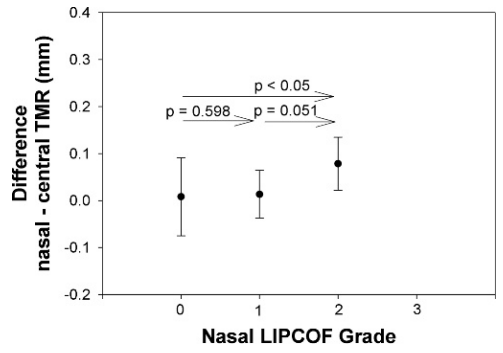

FIGURE 9.
Mean difference between the nasal and central TMR in the four subgroups with different LIPCOF grades.

TABLE 3.
Ocular Surface Disease Index scores and LIPCOF grades for the asymptomatic and symptomatic subjects

	Asymptomatic (n = 26)	Symptomatic (n = 16)
OSDI score, mean ± SD	5.99 ± 3.61	18.26 ± 5.28*
Temporal LIPCOF grade, mean ± SD	1.16 ± 0.73	1.81 ± 0.91*
Nasal LIPCOF grade, mean ± SD	0.58 ± 0.81	0.56 ± 0.81
Temporal LIPCOF: grade 0/ grade 1/grade 2/grade 3, n	5/12/9/0	0/8/3/5
Nasal LIPCOF: grade 0/grade 1/ grade 2/grade 3, n	16/5/5/0	10/3/3/0

*Statistically significant difference (p < 0.05).

fluorescein is added to the tear film, which in turn renders the test invasive and will introduce errors. In contrast, TMR measurement is noninvasive and because the radius is measured, there is no need to detect the upper limit of the meniscus.

The PDM was also able to measure TMR, for the first time, at different locations along the lower lid. In previous studies, a significant positive correlation has been reported between TMH and TMR at the central position; thus, a steeper TMR can be expected in eyes with lower TMH, whereas a flatter TMR correlates with higher TMH.[33,34] In this study, a flatter TMR was found at the temporal and nasal position compared with the central position, which concurred with the higher values of TMH found at these locations.

In contrast to these findings, Jones et al.[8] reported that central TMH was significantly greater than that found in the nasal and temporal areas 3 mm from the nasal and temporal canthi. These differences may be principally explained by the different locations between the two studies. Furthermore, in this study, the measuring time after a blink was controlled (3 to 4 seconds after a blink), whereas it was not controlled in the study by Jones et al. However, Maki et al.[35,36] has shown that, based on a mathematical model, the volume distribution of the tear film changes significantly over time between blinks. Jones at al.[8] hypothesized that gravity forces a pool of tears to form at the center of the lower eye lid, whereas Garcia-Resua et al.[10] hypothesized that tear fluid surface tension may explain the higher values of nasal and temporal TMH.

Harrison et al.[9] showed no significant thinning of the inferior tear meniscus at the limbus compared with the central cornea. However, because they visualized the meniscus with fluorescein and also measured TMH at the area where the lower lid contacts the limbus, it is inappropriate to compare their results with our findings.

Observed temporal and nasal LIPCOF degrees in this study are in concordance with previously reported LIPCOFs.[15,37,38] Lid-parallel conjunctival folds are small folds of the lower bulbar conjunctiva, parallel to the lower lid margin. Lid-parallel conjunctival fold scores have been reported to be increased in dry eye, but they are not age related,[39,40] whereas conjunctivochalasis has been defined as the redundant, loose, nonedematous conjunctival tissue found at the lower eyelid, typically in older people.[17,41] The temporal LIPCOF score in this study was greater in the symptomatic group, which supports earlier findings of LIPCOF being a good discriminator between normal and dry eye patients.[25,42]

Because LIPCOF and conjunctivochalasis are both located in the area of the tear meniscus, it is possible that they can influence the distribution of tear fluid along the lower eyelid. Huang et al.[18] found that the conjunctival folds in conjunctivochalasis obliterate tears not only in the meniscus but also in the reservoir, and they assumed that the conjunctival folds could occupy and deplete the tear reservoir in the fornix. Conjunctivochalasis is often used to describe more prominent folds than described by LIPCOF, which are around 0.08 mm in height.[26]

The severity of conjunctival folds can be affected by the status of contact lens wear. This effect is thought to be an immediate mechanical effect of the contact lens[15] or a long-term effect caused by an increased friction attributed to tear film instability.[37] Although the subjects in this study were not allowed to wear contact lenses during the procedure and for 2 weeks before the study, an immediate effect can be negated. It is possible that a long-term effect of contact lens wear might have influenced the LIPCOF grades.

Using OCT images, Veres et al.[43] observed the coverage of LIPCOF by the tear meniscus and hypothesized that, after a blink, there is a coverage of the conjunctival folds by the tear film. However, in this study, an irregularity of TMH and TMR was found with LIPCOF grades 2 and 3. Therefore, one hypothesis may be that LIPCOFs in the tear meniscus act as a barrier to the normal flow of tears along the lower eyelid (tear flows along the lower lid margin from the temporal side toward the punctum and takes about 3 seconds after blink)[1,9] and that this impedance to the tear flow produces an increase in the tear volume at the temporal and nasal location of the LIPCOFs (Fig. 10). A similar idea was previously described by Guillon.[44] He argued that LIPCOFs might affect the morphology of the reservoir, which loses its meniscus shape and follows the contour of the underlying conjunctiva.

Holly and Lemp[45] reported that a scanty or discontinuous inferior tear meniscus was indicative of an aqueous tear deficiency or lipid abnormality. Taylor[46] described the inferior tear meniscus as ''intact,'' ''not intact temporally,'' or ''not intact'' and found the marginal tear strip continuity to be a method of assessing the adequacy of the tear film. Guillon[44] reported that the reservoir may be interrupted and that this is one sign of potential dry eye symptoms.

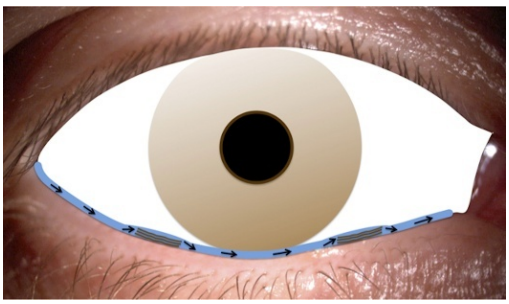

FIGURE 10.
Barrier hypothesis for an irregular tear meniscus along the lower lid: the LIPCOF in the tear meniscus act as a barrier, and tear flow from the outer to the inner canthus is impounded at the temporal and nasal location of the folds. A color version of this figure is available online at www.optvissci.com.

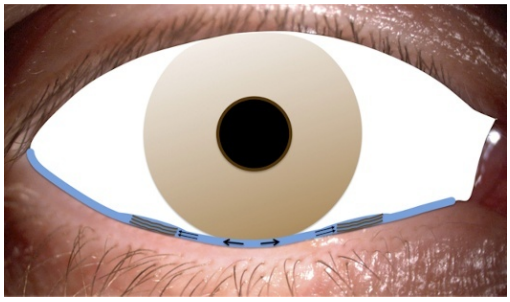

FIGURE 11.
Capillary hypothesis for an irregular tear meniscus along the lower lid: the small distance between two LIPCOF generates capillary forces that draw the surrounding tear fluid toward the folds after a blink. A color version of this figure is available online at www.optvissci.com.

A subjective classification of tear meniscus profile was suggested by Khurana et al.[47] and modified by Garcia-Resua et al.[10] Grades 1 and 2 represent a healthy meniscus, whereas grades 3 and 4 represent an abnormal meniscus.

When comparing the change in central lower TMH immediately after a voluntary blink with TMH 3 seconds after the blink, Veres et al.[43] observed an almost 10-fold higher central tear volume decrease in patients with multiple conjunctival folds than in patients with single folds. They assumed that a sharp decrease in tear volume occurs after blinking in the neighborhood of the multiple folds. This appears to agree with this study showing that, in the presence LIPCOF scores greater than 1, a smaller central TM is produced, compared with temporal or nasal TM, when measurement was performed 3 to 4 seconds after a blink. On the basis of this, we can speculate that, following a blink, the tear flow may be driven from the central to the temporal and nasal LIPCOF area, leading to a central decrease and temporal/nasal increase of TM. It may be hypothesized that the small distance between two conjunctival folds generates capillary forces drawing tear fluid toward the folds (Fig. 11). This force might be more strongly generated if there is more than one fold, which would explain the alteration in TM with LIPCOF grades of greater than or equal to 2 as analyzed in this study.

CONCLUSIONS

In summary, the PDM is able to noninvasively measure alterations in TMR and TMH along the lower lid. The flatter TMR and higher TMH at the nasal and temporal locations may be caused by the LIPCOF degree of the underlying conjunctiva. To avoid any interference by LIPCOF, it is recommended that TMR and TMH are measured along the lower lid margin below the pupil center.

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2.4. Time Course of Changes in Tear Meniscus Radius and Blink Rate after Instillation of Artificial Tears

Cornea

Time Course of Changes in Tear Meniscus Radius and Blink Rate After Instillation of Artificial Tears

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Purpose. Using a novel digital meniscometer (PDM), alterations in tear meniscus radius (TMR) were measured simultaneously with blink rate (BR) following the instillation of artificial tears.

Methods. Central TMR and BR of 22 subjects (11 male and 11 female; mean age, 24.3 ± 2.6 SD years) were measured at baseline, and 0, 1, 5, 10, and 30 minutes after instillation of an artificial tear containing hydroxypropyl-guar and glycol (SYS) or saline (SAL). A dose of 35 µL was applied in one eye in a randomized order with a washout period between each drop.

Results. For SAL, compared to baseline TMR (0.33 ± 0.08 mm), TMR significantly increased with drop instillation (1.55 ± 0.69 mm) and at 1 minute (0.66 ± 0.36 mm; $P < 0.05$), but returned to baseline after 5 minutes. For SYS, TMR (0.32 ± 0.07 mm) remained significantly increased after application (1.62 ± 0.81 mm), and at 1 minute (0.81 ± 0.43 mm) and 5 minutes (0.39 ± 0.08 mm; $P < 0.05$). Compared to baseline BR with SAL (14.8 ± 7.7) and SYS (14.9 ± 9.4), values were significantly increased upon drop instillation (22.5 ± 11.8; 21.3 ± 11.8; $P < 0.05$), but returned to baseline after 1 minute. Dry eye symptoms were correlated with baseline BR ($r = 0.550$, $P = 0.008$).

Conclusions. Results indicate that PDM can detect changes in TMR following instillation of artificial tears. Difference in residence time reflects the different viscosity of each drop. An overload with a large drop may result in an initially increased BR.

Keywords: tear meniscus, artificial tears, portable digital meniscometer, blink rate, tear volume loss, dry eye symptoms

Tear fluid, produced by the secretory system, is distributed and mixed with the preocular tear film and menisci with each blink and then lost by evaporation, absorption, and drainage from the menisci through the nasolacrimal passage.[1] Normal tear film dynamics requires a balance between production and elimination of tears from the eye.[2] The lacrimal secretory rate and tear meniscus radius (TMR) are related to tear volume.[3–5] Blinking is important for the distribution and drainage of the tear fluid.[6–8] The blink rate (BR) is influenced by various factors, such as ocular irritation, precorneal tear film condition, visual demands, or environmental conditions.[9–11]

Artificial tears are used commonly to increase tear volume and retention, and to improve tear film quality. The retention time of instilled fluids, like artificial tears, has been studied with different techniques, such as dacryoscintigraphy, reflective meniscometry, or optical coherence tomography (OCT).[12–16] However, the impact of different solutions on the time course of changes in BR and simultaneously on the change in tear volume remains unknown.

Recently, an iPod Touch–based system (Apple, Inc., Cupertino, CA, USA), named the Portable Digital Meniscometer (PDM), has been developed to measure TMR. It has been demonstrated as giving accurate and reliable measurements at the central position, which were correlated significantly with OCT and video-meniscometer values.[17,18] Furthermore, the PDM has shown the capability to detect variations in TMR along the lower lid.[19] However, it is not known how effective this new system is at assessing TMR changes after the instillation of artificial tears.

The aims of this study were to investigate the capability of a novel slit-lamp mounted, PDM to measure alterations in TMR after the instillation of artificial tears, and to evaluate any relationships between TMR alterations and changes in BR.

Materials and Methods

Subjects

We recruited 22 healthy subjects (mean age, 24.3 ± 2.6 SD years; male = 11, female = 11) from the staff and students of the Höhere Fachschule für Augenoptik Köln (Cologne School of Optometry), Cologne, Germany. Subjects were excluded if they were pregnant or breast-feeding; had a current or previous condition known to affect the ocular surface or tear film; had a history of previous ocular surgery, including refractive surgery, eyelid tattooing, eyelid surgery, or corneal surgery; had any previous ocular trauma; were diabetic; were taking medication known to affect the ocular surface and/or tear film; and/or had worn contact lenses during the preceding two weeks before the study. Cosmetics use was avoided before the procedure. All subjects gave written informed consent before participating in the study. The procedures obtained the approval of the Cardiff School of Optometry and Vision Sciences Human Ethics

Committee and were conducted in accordance with the requirements of the Declaration of Helsinki.

Ocular Surface Disease Index (OSDI)

Each subject's symptoms were evaluated before the application of the drop using the OSDI questionnaire, and afterwards the total OSDI scores were calculated.[20] Analysis of OSDI was masked against TMR and BR measurements.

TMR Measurement

A newly developed slit-lamp mounted PDM was used to measure the central TMR at the lower eyelid. The PDM is based on an application that creates a series of black and white gratings on the screen of an iPod Touch or an iPhone (Apple, Inc.). The PDM is positioned close to and in front of the eye, and the lower lid tear meniscus acts as a concave mirror, creating an image of the grating (Fig. 1A).[3] This image, when captured or recorded by a digital slit-lamp camera (BQ900 with IM900 digital imaging module; Haag-Streit, Koeniz, Switzerland), can be analyzed using ImageJ 1.46 software (available in the public domain at http://rsbweb.nih.gov/ij) (Fig. 1B). The detailed construction of the PDM has been described previously.[17,18]

With the PDM, a 30-second film of the meniscus was recorded using the digital slit-lamp at baseline, and 0, 1, 5, 10, and 30 minutes after instillation of either an artificial tear containing hydroxypropyl-guar and glycol (Systane Balance [SYS]; Alcon Laboratories, Inc., Fort Worth, TX, USA) with a viscosity of 42 cP, or an isotonic sodium chloride solution (SAL, Lens Plus OcuPure; Abbott Medical Optics, Inc., Santa Ana, CA, USA), viscosity 1 cP. Using a micropipette (Pipetman; Gilson S.A.S., Villiers-le-Bel, France), a defined drop size of 35 µL was applied in the temporal lower fornix of the right eye. This drop size represents an average of ophthalmic solution drop sizes,[21,22] and was used previously in similar studies.[8,12–14] The drops were applied in a randomized order with a washout period of at least 1 week between the different solutions. Care was taken to avoid overspill when applying the drop. An image for analysis, at each time point, was captured from the recorded video of the meniscus two seconds after a spontaneous blink when a stable image was achieved. The images then were exported to ImageJ where TMR was measured.

Blink Measurement

Each recorded 30-second sequence of subject blinking, at each time point, was viewed in a ×0.25 slow-motion mode with the VLC Media Player 2.06 (available in the public domain at http://www.videolan.org/vlc), and the BR per minute analyzed at baseline, and at 0, 1, 5, 10, and 30 minutes after instillation of the different solutions.

The study was conducted in a room with controlled temperature (20°C–23°C) and humidity (44%–53%). All measurements of the lower tear meniscus radius and BR were taken on the right eye in primary gaze by a single observer. Analysis of tear meniscus radius was masked against BR count. The examiner was masked to the different drops and time points. To minimize diurnal variation, images were recorded in the morning between 10 and 12 o'clock.

Calculation of Tear Volume Loss (TVL) and Tear Volume Loss Rate (TVLR) per Blink

Total tear volume was calculated by the equation between TMR and tear volume, which was described previously by Yokoi et al.[4]:

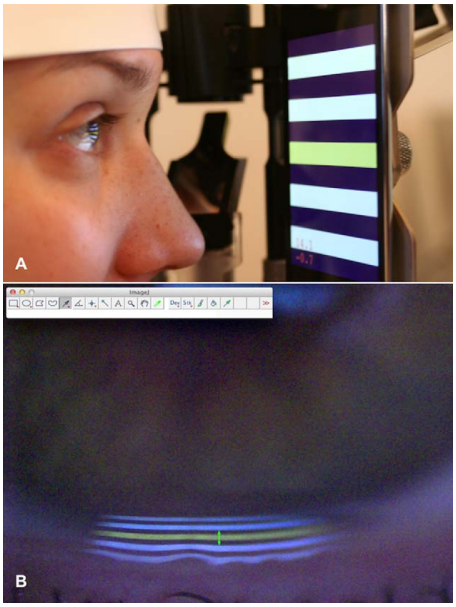

FIGURE 1. (**A**) Patient positioned in front of the portable, slit-lamp mounted PDM. The grid on the screen of the iPod Touch is reflected by the cornea and the lower tear meniscus. (**B**) ImageJ 1.46 software for measurement of line distance on the PDM image.

$$Total\ Tear\ Volume\ (\mu L) = [(TMR - 0.256)/0.038] + 6.7$$

The TVL was calculated for both solutions for the time intervals between 0 and 1 minute, 1 and 5 minutes, 5 and 10 minutes, and 10 and 30 minutes after the applications. To calculate the TVLR per blink in the different time intervals, the TVL was divided by the BRs that were analyzed for the relevant time interval.

Statistical Methods

Data were tested for normality using the Shapiro-Wilk test. The time course of changes in TMR and BR was statistically analyzed using 1-way ANOVA on ranks (Kruskal-Wallis test). If significant differences were observed, a Dunnett post hoc test for multiple comparisons was performed to find time points showing a significant difference to the baseline value. Differences between the test solution effects on TMR and BR at various time points were analyzed by the paired *t*-test (for normal distribution) and Wilcoxon signed ranks test (for non-normal distribution). Correlations between BR and OSDI score were evaluated by Spearman rank order correlation. The data were analyzed using SigmaPlot 12 (Systat Software, Inc., Chicago, IL, USA).

RESULTS

Changes in TMR

Compared to baseline values (0.33 ± 0.08 mm) TMR with SAL was significantly increased upon application of drop (1.55 ±

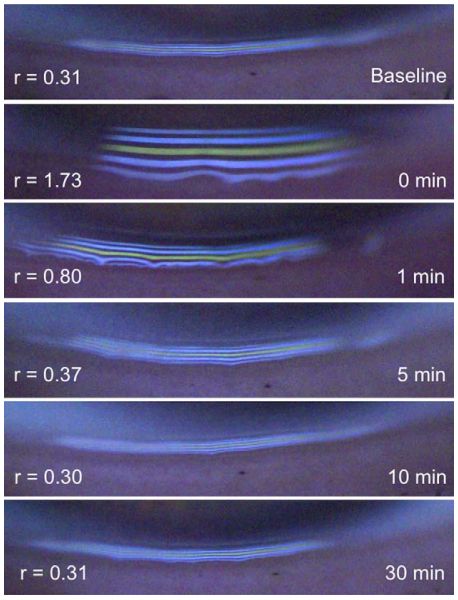

FIGURE 2. Representative PDM images of the dynamic changes in the lower TMR before and after instillation of artificial tears containing SYS.

0.69 mm) and remained significantly greater at 1 minute (0.66 ± 0.36 mm; ANOVA on ranks with Dunnett post hoc test, $P < 0.05$), but became similar to baseline after 5 minutes (0.34 ± 0.08 mm; $P = 0.417$). In contrast, TMR with SYS (baseline TMR, 0.32 ± 0.07 mm) remained significantly increased after application (1.62 ± 0.81 mm), up until 1 minute (0.81 ± 0.43 mm) and 5 minutes (0.39 ± 0.08 mm; $P < 0.05$, Fig. 2). Compared to SAL, TMR with SYS was significantly flatter at 1 minute (0.15 ± 0.32 mm; $P = 0.044$) and 5 minutes (0.05 ± 0.08 mm; $P = 0.008$, Fig. 3). For all other points in time there was no significant difference between the two solutions.

Changes in BR

Baseline BRs with SAL (14.8 ± 7.7) and SYS (14.9 ± 9.4) were significantly increased upon application of drops (22.5 ± 11.8 and 21.3 ± 11.8; ANOVA on ranks with Dunnett post hoc test, $P < 0.05$), but became similar to baseline figures after 1 minute ($P > 0.05$, Fig. 4). For all points in time there was no significant difference in BR between the two solutions.

TVL and TVLR per Blink

The calculated TVL of SAL and SYS in the different time intervals is summarized in the Table.

For both solutions there was no statistically significant difference in the calculated rate of TVL per blink when comparing the first time interval 0 to 1 minute (SAL, 1.24 ± 1.16; SYS, 1.41 ± 1.72 µL/blink) to the second time interval 1 to 5 minutes (SAL, 0.68 ± 1.03; SYS, 0.83 ± 0.79 µL/blink) and the third time interval 5 to 10 minutes (SAL, 0.02 ± 0.11; SYS, 0.12 ± 0.12 µL/blink) to the fourth interval 10 to 30 minutes (SAL 0.07 ± 0.17; SYS 0.08 ± 0.23 µL/blink; ANOVA on ranks

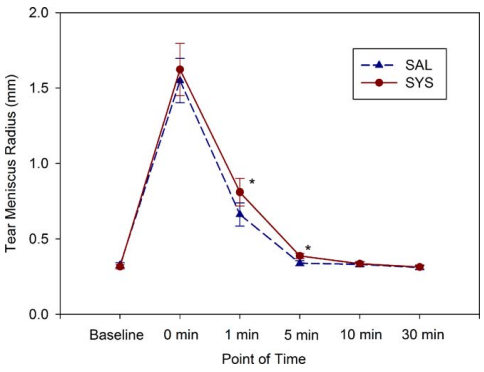

FIGURE 3. Variations in TMR after the instillation of artificial tears. *Indicates a statistically significant difference between the two solutions (paired *t*-test, $P < 0.05$). Values are mean ± SE.

with Dunnett post hoc test, $P < 0.05$). The comparison between all other time intervals (first to third and fourth intervals, and second to third and fourth intervals) showed a statistically significant difference in the rate of TVL per blink ($P < 0.05$, Fig. 5).

Correlation Between OSDI and BR

Mean OSDI score at baseline was 10.5 ± 7.7 (SD) with a range from 0 to 27.1. The OSDI score was correlated with the BR at baseline (Spearman's rank correlation coefficient, $r = 0.550$; $P = 0.008$; Fig. 6).

DISCUSSION

We reported the use of a new custom-made PDM to evaluate the dynamic changes of the lower TMR after adding artificial tears. Using the PDM, an increase in TMR (and, therefore, tear

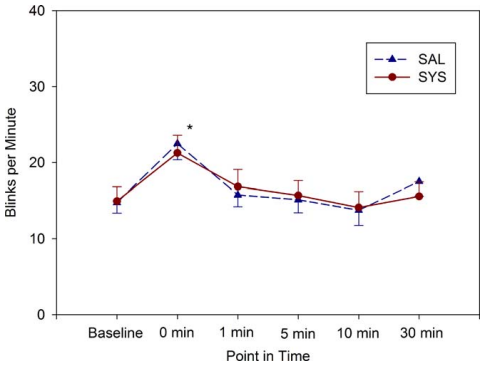

FIGURE 4. Variations in BR after the instillation of artificial tears. *Indicates a statistically significant difference from the baseline values (ANOVA on ranks with Dunnett post hoc test, $P < 0.05$). Values are mean ± SE.

TABLE. Calculated TVL in Microliters (Mean ± SD) in the Different Time Intervals

Time Interval, µL	0–1 Min	1–5 Min	5–10 Min	10–30 Min	Total
SAL	−23.3 ± 16.5	−11.0 ± 9.0	−0.1 ± 1.0	−0.4 ± 1.5	−34.8 ± 17.8
SYS	−21.4 ± 16.6	−11.3 ± 10.5	−1.4 ± 1.3*	−0.6 ± 1.5	−34.5 ± 22.3

* Indicates a statistically significant difference between the two solutions (paired *t*-test, $P < 0.001$).

volume) was found after instillation, with a return to baseline figures after 5 minutes for the SAL solution and after 10 minutes for the artificial tears containing SYS.

Wang et al.[12,14] measured the dynamic changes of tear meniscus height (TMH), TMR, and tear meniscus cross-sectional area (TMA) after artificial tear instillation using a custom-made OCT system. They found the tear meniscus parameter returned to baseline 5 minutes after instillation of saline (viscosity 1 cP), carboxy-methylcellulose sodium (CMC) 0.5% and 1.0% (3 and 70 cP), and propylene glycol 0.3% (10 cP). However, they found an increase in tear film thickness and lower tear meniscus variables at instillation with the more viscous drops in healthy patients. Also using CMC in a concentration of 0.5% and 1.0% in dry eye patients and controls, Wang et al.[13] used a spectral domain OCT to measure TMH and TMA changes. While in the control group the 0.5% and 1% CMC persisted for 1 and 15 minutes, in the dry eye group the artificial tears persisted for 5 and 30 minutes. They suggested that the longer retention time is associated with the viscosity of the drop and, furthermore, that in a dry eye patient, a lower tear clearance rate might prolong the retention time. In this study, when measuring TMR with the PDM in subjects without significant dry eye, a two times longer retention time was found with the more viscous drop compared to saline. Although in this group the differences between the drops were small, but statically significant, a clinically more relevant difference could be expected in dry eye patients, as suggested by Wang et al.[12] Interestingly, the difference of 0.05 mm in TMR after 5 minutes represents a difference in volume of 1.3 µL (see Table). Estimating a total tear volume of 6.2 µL,[5] this represents an increase of approximately 20%, which might be clinically relevant.

Furthermore, the artificial tears we used in this study were formulated specifically to minimize the evaporative loss of tears from the ocular surface, by adding a polar phospholipid surfactant and mineral oil.[23] It is possible, therefore, that a difference in tear evaporation rate between the two drops used will have impacted the changes in TMR.

Yokoi et al.[4] investigated the relationship between tear volume and TMR measured using a video-meniscometer, concluding that there is a linear relationship between the volume of the instilled saline solution and the measured TMR. Applying the video-meniscometer, they showed that a 0.1% hyaluronic acid solution resided longer in the tear meniscus than a solution containing 0.1% KCl and 0.4% NaCl.[15] The PDM in this study is based on the video-meniscometer,[3] where the tear strip acts as a concave mirror, and, likewise, we were able to detect changes in tear volume by measuring the dynamics of TMR.

Besides the volume and the viscosity of the drop, blinking has an important role in the distribution and drainage of instilled fluid. The lacrimal drainage capacity in young individuals was correlated with the BR.[7] Palakuru et al.[8] analyzed the blink outcome, defined as the difference in tear volume before and after a blink, upon the instillation of 35 µL of 1% CMC. Immediately after the drop was applied, the blink outcome of one blink was increased compared to the blink outcome after five minutes. They concluded, that the increase in blink outcome helps to restore balance when the instilled drop overloads the tear system. Zhu and Chauhan[24] used a mathematical model, and calculated a drainage rate of 1.174 µL per blink for the overloaded tear film. Overloading the tear film by repeatedly instilling saline solution into the tear film for 3 minutes, Sahlin et al.[25] reported drainage rates of 1.11 to 4.03 µL per blink. In this study, the volume loss rates of 1.24 and 1.41 µL per blink in the first time interval of 0 to 1 minute are in good agreement with the previously reported values. Interestingly, even though the tear volume after 1 minute was significantly diminished, the volume loss rate per blink in the second interval (1–5 minutes) was not significantly different from that in the first interval. This fact might be explained by the observation of an increase in BR upon

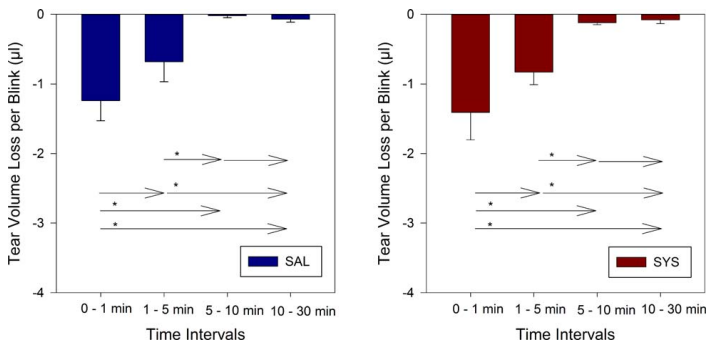

FIGURE 5. Calculated TVL per blink in the different time intervals after the instillation of a 35 µL drop. *Indicating a statistically significant difference between the time intervals (ANOVA on ranks with Dunnett post hoc test, $P < 0.05$). Values are mean ± SE.

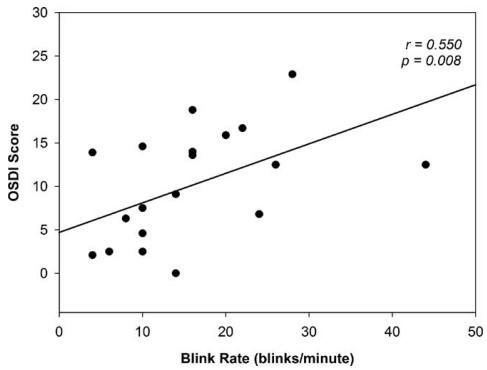

FIGURE 6. Relationship between OSDI score and BR at baseline. (Spearman's rank correlation coefficient, $r = 0.550$, $P = 0.008$).

application of drops with a return to baseline after 1 minute. These results favor the interpretation that, during the initial overload phase, the increase in tear volume results in an increase in BR, but that as soon as the volume is reduced to a certain level, a reduction in BR keeps the volume loss rate per blink nearly constant. Once the overload is removed, the volume loss rate per blink of the normal tear film stays constant (Fig. 5). This mechanism has not been reported previously to our knowledge, although Palakuru et al.[8] argued for a relationship between tear volume and BR output based on the analysis of a single blink.

The spontaneous BR at baseline in this study compares well with the literature.[26,27] Upon drop instillation, the BR increased with no difference in the BRs between the two solutions. Based on these observations, we hypothesized that the viscosity of the drop does not seem to influence the effect. However, the difference in viscosities of the two drops used in this study may be too small and the variations in BRs too large to detect an effect of drop viscosity on BR.

Dry eye patients exhibit an increased BR in response to the drying of the ocular surface.[9,10,28] Although the cohort in this study was very young, we confirmed a correlation between symptoms evaluated by the OSDI scores and the BRs.

A limitation of this study may be that completeness of blink was not assessed. Recent studies suggest that not only the frequency, but also the completeness of blink may have an effect on dry eye symptoms.[26,29] Further studies are needed to examine the effect of different types of blinking on the loss of tear film volume.

In summary, the PDM is able to detect changes in TMR usefully following the instillation of artificial tears. The difference in residence time is likely to reflect the different viscosity and Newtonian properties of these drops. An overload with a large drop may result in an initial increased BR. The BR at baseline was significantly related to dry eye symptoms.

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