PARACELSUS
MEDIZINISCHE PRIVATUNIVERSITÄT

"Introducing a new parameter for the Assessment of the Tear Film Lipid Layer"

Dissertation zur Erlangung des akademischen Grades

Doktor der medizinischen Wissenschaft (Dr. scient. med)

an der Paracelsus Medizinischen Privatuniversität, Salzburg

eingereicht von

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am „21.11.2012"

unter Betreuung von

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Inhalt

1 Background

This first chapter gives general information on the importance of the pre-ocular tear film. The relevance is further underlined by epidemiological data of Dry Eye Disease (DED). To point out the complexity of this fluid, a short description of the main purposes of the tear fluid is additionally provided.

1.1 Dry Eye Disease

One of the most common complaints of patients visiting ophthalmologists are symptoms of ocular discomfort, like dryness or grittiness as a result of an unstable tear film. The diagnosis of an insufficient tear film is often referred to as Dry Eye Disease (DED) or keratoconjunctivitis sicca (KCS), which can be considered as a subgroup of ocular surface diseases (Lemp 1984). Estimations in order to quantify the burden of this disease state that an absolute number of 3.23 million female Americans compared to 1.68 million male Americans at an age of 50 years and older are affected by an impaired tear film and the after-effects of this constitution (Schaumberg, Sullivan, et al. 2003) (Schaumberg, Dana, et al. 2009).

The global prevalence of DED ranges between 5% (McCarty, et al. 1998) and 35% (Lin, et al. 2003). These differences can partly be explained by the different assessment procedures and diagnostic criteria (objective clinical parameters versus subjectively reported symptoms). Ethnicity, age and gender additionally contribute to the high variation of these parameters.

The strong relation of the constitution of the pre-ocular tear film, with the ocular surface health and with subjectively reported symptoms can be explained by considering the main purposes of the tear film.

1.2 Main purposes of the tear film

1.2.1 Lubrication of the ocular surface

Probably the most obvious purpose of the tear film is the wetting and lubrication of the ocular surface. This is pivotal for the reduction of shear forces acting on the underlying corneal epithelia during the closing and opening phase of the blink activity. The wetting of

the ocular surface is performed by two different types of tearing: basic and reflex tearing, both with different nervous innervation, purpose and capacity.

Basic tearing is mainly fulfilling the aforementioned purpose. It has been suggested that this steady production of tears is performed in combination of both the main and the accessory lacrimal glands (Chapter 2.3).

Reflex tearing is induced by emotional or physical stimulation of the lacrimal gland, e.g. to remove foreign substances which could potentially mechanically harm the ocular surface.

To maintain a continuous regeneration of the corneal epithelia, tears contain factors for the rebuilding of the corneal epithelia, like vitamin A and growth factors (van, et al. 1989) (Ohashi, et al. 1989) ,Chapter 2.2.3).

1.2.2 Smoothness of the optical surface

Since the tear film is the first refractive barrier for the incoming light, transient changes in the visual perception are a commonly reported problem of patients with DED. It is supposed that an inhomogeneous distribution of the tear film is responsible for such short term impairments of vision following a blink action (Benito, et al. 2011) (Ridder, et al. 2011).

Especially the outermost layer of the tear film, the tear film lipid layer, has been reported to be crucial for providing a smooth optical surface for the incoming light. Variation in thickness of this layer along the exposed corneal surface resulting in different refractive indices and chromatic aberration could be a possible explanation for the transient visual impairment of many DED patients (Bron, et al. 2004) (Begley, Chalmers, et al. 2003).

1.2.3 Immune defense mechanisms

Since the eye is a potential portal of entry for pathogens, immune defense mechanisms are incorporated immune defense mechanisms. Specific proteins therefore can be found mainly in the soluble environment of the middle and thickest layer of the tear film, the aqueous layer. The main antimicrobial proteins are shown in Table 1.

Protein	Concentration
Lactoferrin	2.2 mg/ml (Flanagan und P 2009)
Lysozyme	2.5 mg/ml (Wiesner und Vilcinskas 2010)
Tear Lipocalin	2 mg/ml (Dartt 2011)
IgA	0.8 mg/ml (Masinick, et al. 1997)

Table 1: Overview of the major antimicrobial proteins (Zhou und Beuerman 2012)

Lactoferrin is mainly acting against microbial activity by binding iron to pathogens, which they need for growth and pathogenesis. Furthermore this protein provides protection by preventing entering viruses from binding to the corneal epithelia and by the interference of the viral pathogenesis.

Lysozyme causes the disruption of the cell walls of especially Gram-positive bacteria, by the hydrolysis of peptiodoglycan, an essential layer of the cell membrane. Likewise Gram-negative bacteria are attacked by lysozyme. It is proposed that possible electrostatic interactions are the reason for this immune response.

Secretory Immunoglobulin A (sIgA) represents an antigen specific response. It thereby binds to surface antigens of bacteria cell walls thus inhibiting the adherence of these microbes to the corneal surface.

The contribution of lipocalin to the immune response is the binding to microbial siderophores, which are secreted by bacteria in order to bind and deliver iron back to the microbe (Zhou und Beuerman 2012).

1.2.4 Support corneal metabolism

The cornea is organized in different layers as can be seen in Figure 1, resulting in a central thickness of about 0.5 to 0.6 mm and a peripheral thickness of 0.6 to 0.8 mm.

To ensure optimal optical performance the corneal tissue has to be translucent. To achieve this, the cornea largely consists of collagen and few cells, no connective tissue and almost no vessels. The latter fact makes it necessary that the layers have to be provided with nutrients through the process of diffusion. To maintain a sufficient metabolism the cornea has to be provided with mainly glucose and oxygen.

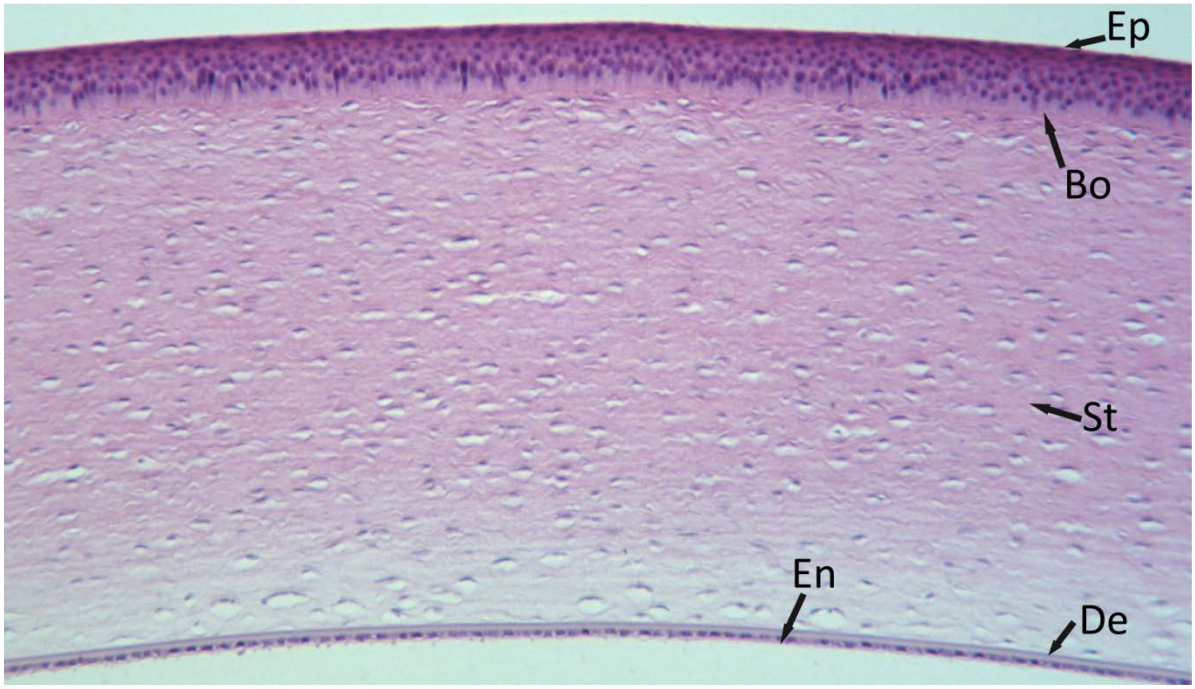

Figure 1: Structure of the corneal tissue; (Ep)ithelium, [Bo]wman's layer, (St)roma, (De)scemet's membrane, (En)dothelium; http://www.rutilioculista.it/anatomia-fisiologia/cornea/

The nutrients and waste products are transported to and from the cornea by the pre-ocular tear film and the aqueous humor.

Three mechanisms exist to ensure a proper metabolism:

[a] In the presence of oxygen the way of the aerobic glycolysis, the resulting waste products are thereby carbon dioxide and water.

[b] Without sufficient oxygen the metabolism is based on anaerobic glycolysis. Lactate acids are the main resulting waste products.

[c] The transformation of glucose to ribose is the third way. The resulting monosaccharide pentose is further on crucial for the biosyntheses of nucleic acids (RNA, DNA) and coenzymes (ATP).

2 Structure of the tear film

This chapter provides the reader with information on the basic structure of the tear film.

In general the tear film can be considered to consist of three layers: the innermost mucus layer, the aqueous layer and the lipid layer. This clear separation of the different layers is more a hypothetical but feasible model to explain the functionality of the tear film (Prydal und Campbell 1992) Figure 2. The average thickness of the pre ocular thickness is estimated to be between 3 and 40 µm, dependent on the different methods for the assessment of the tear film (King-Smith, Fink und Fogt 1999) (Maurice 1973) (Prydal und Campbell 1992).

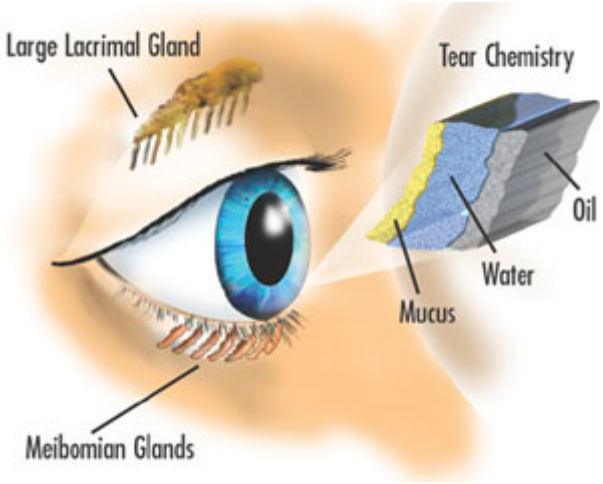

Figure 2: Principal structure of the pre ocular tear film http://www.eyehealthcare.net/symptoms-of-dry-eyes.html

It is important to notice that a number of important interactions occur between these layers, making it hard to perform such a distinct separation of the different components based on functionality.

Worth mentioning is also the fact that each of the layers is produced from different tissues and transported to the ocular surface through separate ways. This fact leads to the conclusion that the secretion processes have to have an underlying, common regulation loop in order to ensure a coordinated performance (Stern, et al. 2004).

2.1 Mucous Layer

The innermost mucous layer has reported to have a thickness of about 1 µm (Nichols, et al. 2002). Mucins can be considered as large glycoproteins as major components of in all mucus secretions of wet-surfaced epithelium. According to their biophysical behavior, mucins can be divided into three groups: transmembrane mucins forming the glycocalix of the corneal

epithelial tissue; gel forming mucins and soluble mucins mainly found in the intermediate, aqueous layer of the tear film. An overview of the mucins can be found in Table 2.

Mucin type	Designation
Gel forming	MUC2
	MUC5AC
	MUC5B
	MUC6
Soluble	MUC7
	MUC9
Transmembrane	MUC1
	MUC3A
	MUC3B
	MUC4
	MUC12
	MUC13
	MUC16
Unknown	MUC8
	MUC11

Table 2: Mucins occuring the the human pre ocular tear film (Argüeso und Gipson 2001)

The glycocalyx of the ocular surface epithelia is mainly formed by transmembrane mucins MUC1, MUC4 and MUC16. These mucins ensure the wettability of the ocular surface and interact with gel forming mucins of goblet cell origin (Bron, et al. 2004).

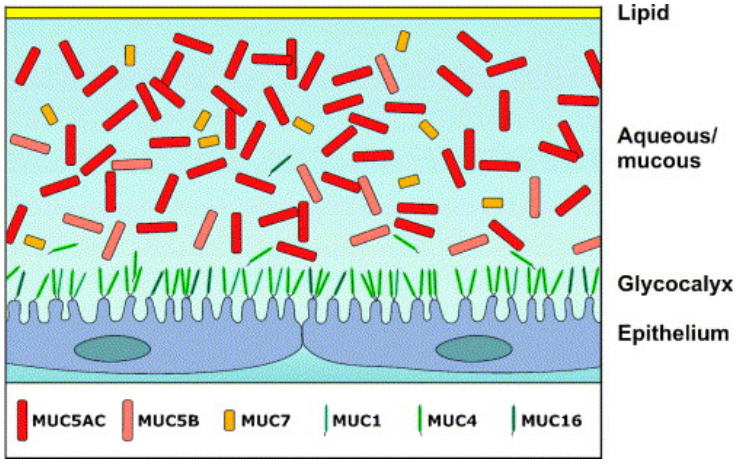

Figure 3: A diagram illustrating a hypothesis of the structure of the tear film on the ocular surface (Paulsen 2006)

The purpose of the mucins is discussed controversially. Holly and Lemp proposed that the mucins lower the surface tension of the otherwise non-wettable surface. Also the non-

Newtonian viscosity of the tear film which plays a crucial role in the lubrication of the ocular surface was initially contributed to the mucins (Holly und Lemp, Tear physiology and dry eyes. 1977) (Holly und Lemp, Wettability and wetting of corneal epithelium. 1971). In addition, MUC7 is known to have antimicrobial activity in the salivary gland. It is assumed that these mucins also fulfill this functionality within the lacrimal gland.
Meanwhile, it has been questioned that the low concentration of MUC5 can be responsible for the lowering of the surface tension since a concentration of 0.1 to 0.2 % of mucin would be needed to achieve observed values. This concentration of gel forming MUC5AC is usually not achieved in human tears (Pandit, et al. 1999). The proposed alternative influencing the physical properties is the lipid binding lipocalin in the aqueous layer together with lactoferrin and sIgA (Tiffany und Nagyová 2002).

The mucins are expressed in three different places: corneal and conjunctival epithelia; conjunctival goblet cells and in lacrimal apparatus.

At least three of the transmembrane mucins are contributed to the corneal and conjunctival epithelia (MUC1, MUC4, MUC16). MUC1 and MUC16 are both present on corneal and conjunctival epithelia and are known to interact with proteins associated to the cytoskeleton. MUC4 is most prevalent in the conjunctival epithelium with a significant reduction towards the central corneal epithelium.

The gel forming mucin MUC5AC are produced by goblet cells, specialized cells incorporated in the conjunctival epithelium. The highest density of these cells is in the infero-nasal portion of the bulbar conjunctiva. It is still no sure that the gel forming MUC2 is the result of globlet cell specific mechanisms.

The remaining mucins are produced and secreted by cells of the main and/or accessory lacrimal glands and of the nasolacrimal glands.

2.2 Aqueous Layer

The intermediate, thickest layer of the tear film is the aqueous layer and has a thickness of about 4 to 7 µm (Sullivan, Dartt und Meneray 1998). From the biophysical point of view this layer promotes the spreading of the tear film. Additionally the aqueous components serve as a solution for most of the proteins in the tear fluid. Important tear proteins are e.g.

lysozyme, immunglobuline A, lactoferrin, lipocalin etc. A detailed overview of tear fluid proteins can be found in Table 3 and in the review of (Ohashi, Dogru und Tsubota 2006).

(1) Albumin: Von Ebner's gland protein (Tear prealbumin), serum albumin
(2) Transferrin: serotransferrin precursor
(3) Lysozyme: lysozyme C precursor (EC 3.2.1.17) (1,4-beta-N-acetylmuramidase C), contribution of hydrophobic effect to the conformational stability of human lysozyme
(4) Ig: IgA, IgG, IgM, Ig alpha-1 chain C region, Ig alpha-2 chain C region, Ig lambda chain C region, Ig kappa chain C region, Ig heavy chain V-III region BRO/or TEI, immunoglobulin J chain, Ig heavy chain V-I region SIE, Ig mu chain C region, Ig heavy chain variable region, polymeric-immunoglobulin receptor precursor (Poly-Ig receptor) (PIGR)
(5) Cystatin: cystatin SN precursor (salivary cystatin SA-1), cystatin S precursor (salivary acidic protein-1) (Cystatin SA-III), cystatin SA precursor (cystatin S5), cystatin D precursor, cystatin C precursor (neuroendocrine basic polypeptide)
(6) Proline-rich protein: proline-rich protein 1, proline-rich protein 3 precursor, proline-rich protein 4, nasopharyngeal carcinoma-associated proline rich 4, proline-rich protein 5 precursor (proline-rich protein PBI)
(7) Lactoferrin
(8) Lipocalin
(9) Epidermal growth factor (EGF)
(10) Aquaporin 5
(11) a-Defensins
(12) Prolactin-inducible protein
(13) Mammaglobin B
(14) Phospholipase A, membrane-associated
(15) Extracellular glycoprotein lacritin precursor
(16) Lipophilin A precursor (secretoglobin family1D member 1)
(17) Beta-2-microglobulin precursor (HDCMA22P)
(18) Antileukoproteinase 1 precursor (ALP)
(19) Brain-specific angiogenesis inhibitor 3 precursor
(20) Aspartyl aminopeptidase (EC 3.4.11.21)
(21) G-rich sequence factor-1 (GRSF-1)
(22) 5V-AMP-activated protein kinase, catalytic alpha-2 chain (EC 2.7.1.-) (AMPK alpha-2 chain)
(23) Oxygen-regulated protein 1
(24) Clusterin precursor (Complement-associated protein SP-40,40)
(25) Mesothelin precursor (CAK1 antigen)
(26) Endothelial transcription factor GATA-2
(27) Nuclear RNA export factor 1 (Tip associating protein) (Tip-associated protein)
(28) Leucine-rich primary response protein 1 (follicle-stimulating hormone primary response protein)
(29) 60S ribosomal protein L18a
(30) Leucine-rich repeat transmembrane protein FLRT3 precursor
(31) Chloride intracellular channel protein 2 (XAP121)
(32) Basic salivary proline-rich protein 4 allele M
(33) Deleted in malignant brain tumors 1 isoform a precursor
(34) KFLA590
(35) Hypothetical protein
(36) Similar to common salivary protein 1
(37) Phospholipid transfer protein precursor
(38) Hypothetical protein

Table 3: Major components of human tear proteins (Ohashi, Dogru und Tsubota 2006)

2.2.1 Lysozyme

About 30 to 40 % of the tear proteins are made up of lysozyme, which is the most alkaline protein in the tear fluid. Lysozyme is acting as an immune defense mechanism, mainly against gram-positive bacteria. Besides in the tear film, lysozyme can be found in the saliva, in the nasal mucus, in the serum, in the liquor, in the amniotic fluid, in the cervical mucus and in the breast milk. Worth mentioning is the fact that the highest concentration of lysozyme can be found in the tear fluid (0.6 and 2.6 mg/ml) (Avisar, et al. 1979). The concentration is reduced with age and within patients with DED, especially patients with Sjögren syndrome.

Lysozyme is produced by lysosomes and can be considered as a particular long chained glycolytic enzyme. It is acting against bacteria by the enzymatic dissolving of the bacteria's cell membrane, in particular tissue muco polysaccarides. In order to act against Gram-negative bacteria, proteins like lactoferrin first have to make the bacteria's cell wall permeable for lysozyme.

2.2.2 Lactoferrin

Lactoferrin is a multifunctional protein of the family ferrin and, due to the contributions to the immune defense mechanisms, can be found in the saliva, the tears, the nasal secretion and in the breast milk, especially during the late phase of pregnancy.

Considering the pre ocular tear film, Lactoferrin is produced mainly by the acinar cells of the lacrimal gland and accounts for 25 % of the proteins in the tear fluid at an average concentration of about 2.2 mg/ml (Kijlstra, Jeurissen und Koning 1983). Interestingly, the amount of lactoferrin in the tear fluid changes from open to closed state of the eye. Lactoferrin and tear-specific lipocalin are reduced from 85-88 % in the open state to about 30 % in the closed eye state (Willcox, et al. 1997).
Besides in the main lacrimal gland, significant amounts of lactoferrin are also produced by the ocular epithelia with a higher expression within the conjunctival than within the corneal tissue (Tsai, et al. 2006). More recently, proteome analysis of human meibomian gland secretions also identified Lf, suggesting that meibomian glands are also capable of producing lactoferrin (Santagati, et al. 2005).

The purpose of lactoferrin is to act against bacteria (e.g. *staphylococcus epidermidis*) and viruses (e.g. *Adenovirus*). The principal mechanism is the binding of iron and thereby competing with bacterias, which need iron for their metabolism.

The concentration and levels of iron saturation must be finely tuned to effect positive actions of lactoferrin and reduce opportunistic microbial exploitation. A detailed review on the role of lactoferrin in the tear film was published by Flanagan et al (Flanagan und P 2009) .

2.2.3 Epidermal Growth Factor (EGF)

Growth factors in the anterior segment of the eye are mainly responsible for the regulation of cell turnover of the ocular surface. In addition to the pre-corneal tear film, also the aqueous humour contains growth factors in order to control the regenerative processes of the corneal endothelium.

Growth factors in general are soluble peptides, which control the proliferation and/or migration of cells. The corresponding receptor of the target cells are either of the receptor tyrosine kinase (RTK) or of the G-protein-coupled receptor (GPCR) family. Signalling may be induced directly at receptors incorporated in the membrane or through specific ligand-receptor complexes of endosomal parts within the cell membrane (Figure 4).

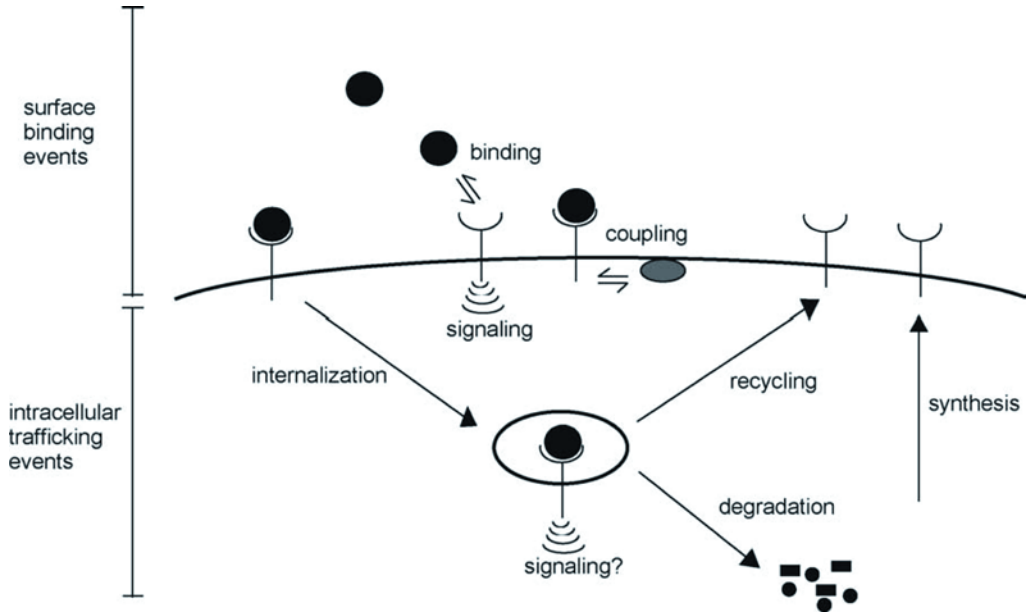

Figure 4: Mechanisms of growth factor action upon receptor binding (Klenkler und Sheardown 2004)

EGF is a potent mitogen for epithelial cell types. Ligand binding to specific receptors within the cell membrane leads to DNA synthesis and cellular proliferation. In addition, the actin cytoskeleton of the target cell is rearranged, leading to an alteration of the motility. EGF has thereby found to inhibit the terminal differentiation of epithelial cells and increase the proliferation between a concentration of 0.1 ng/ml and 10 ng/ml (Hongo, et al. 1992). Levels above leads lead to opposite effects, the proliferation and the cell numbers thereby being reduced.

It has been shown that the functionality of EGF is linked to the presence of other growth factors, in particular fibroblast growth factor (FGF), during the process of wound healing. Other important growth factors incorporated in the ocular surface environment are *Transforming growth factor-β* (TGF- β), inducing proliferation of proliferation and migration of fibroblasts into the corneal stroma (Grant, et al. 1992), *Kerationcyte growth factor* (KGF), inducing DNA synthesis and promotion of corneal epithelial cell grow (Sotozono, et al. 1994), *Hepatocyte growth factor* (HGF), inducing proliferation of both the endothelium and the epithelium of the cornea (Grierson, et al. 2000), *Platelet-derived growth factor* (PDGF), controlling the epithelial cell response to TGF- β (Jester, et al. 2002) and *Fibroblast growth factor*, more prominent in Bowman's and Descement's membranes and in the corneal epithelium, controlling the migration of mainly corneal endothelial cells (Rieck, Cholidis und Hartmann 2001). An extensive review of occurring growth factors in the anterior segment of the eye was published by Klenkler et al (Klenkler und Sheardown 2004).

2.2.4 Immunoglobulin A

Lymphatic B-cells are a main contributor of the humoral immune response mechanism, within they constitute an important part of the adaptive immune system. Those cells are characterized by having a specific antigen receptor incorporated in their cell wall. The main functionality of those Lymphatic B-cells is to produce antibodies, which in turn act against those antigens in different mechanisms:

- Neutralization of the antigen e.g. to block its toxic behavior or prohibit it from interaction with other cells
- Marking the infiltrating cell in order to attract phagocytes which are able to digest the antigen
- Activate complement system of the immune system in order to amplify the aforementioned process of marking the antigens

- Mark antigen-presenting body cells in order to attract natural killer cells to attack them
- Activate the process of agglutination with antigens

Of the various immunoglobulin presented in the tear fluid, IgA is by far the most abundant. It has been shown that the human lacrimal gland contains many antibody secreting cells that mainly stain positive for IgA. Also lymphatic B and T cells, dendritic cells and macrophages are observed. For an extensive review on immunoglobulin classes within the tear fluid was written of Meek et al (Meek, et al. 2003).

2.2.5 Lipocalin

Tear lipocalin (former tear specific albumin) comprises about 15-33 % of the proteins occurring in the pre-ocular tear film (Fullard und Kissner 1991). Lipocalin is produced mainly by lacrimal gland as well as by the salvatory gland of von Ebner (Inada 1984). More recently it has been shown that the human meibomian gland is also capable to express significant amounts of lipocalin (Tsai, et al. 2006).

It is well known that lipocalin has an extraordinary high affinity to lipids. In the specific case of the tear film lipocalin binds to the polar lipids secreted from the meibomian glands (Glasgow, Abduragimov, et al. 1995). An interesting study by Glasgow et al focused on this lipid binding behavior and suggested that tear lipocalin is especially needed as a scavenger for meibom lipids at the corneal surface. It was proposed that a thinning of the tear film, especially during the manifestation of the aqueous deficient deficiency form of DED, lipids making the otherwise hydrophilic corneal surface more hydrophobic, making it less wettable (Glasgow, Marshall, et al. 1999). In addition it has been reported, that lipocalin contributes high, non-Newtonian viscosity of the tear film and its low surface tension (Redl, Holzfeind und Lottspeich 1992).

2.2.6 Lacrimal functional unit

The term lacrimal functional unit covers the ocular surface, the main lacrimal gland and the interconnecting innervation. The ocular surface is thereby defined as the cornea, conjunctiva, accessory lacrimal glands, and meibomian glands. This chapter now describes the principle regulation mechanisms as a response to environmental challenges on the tear film.

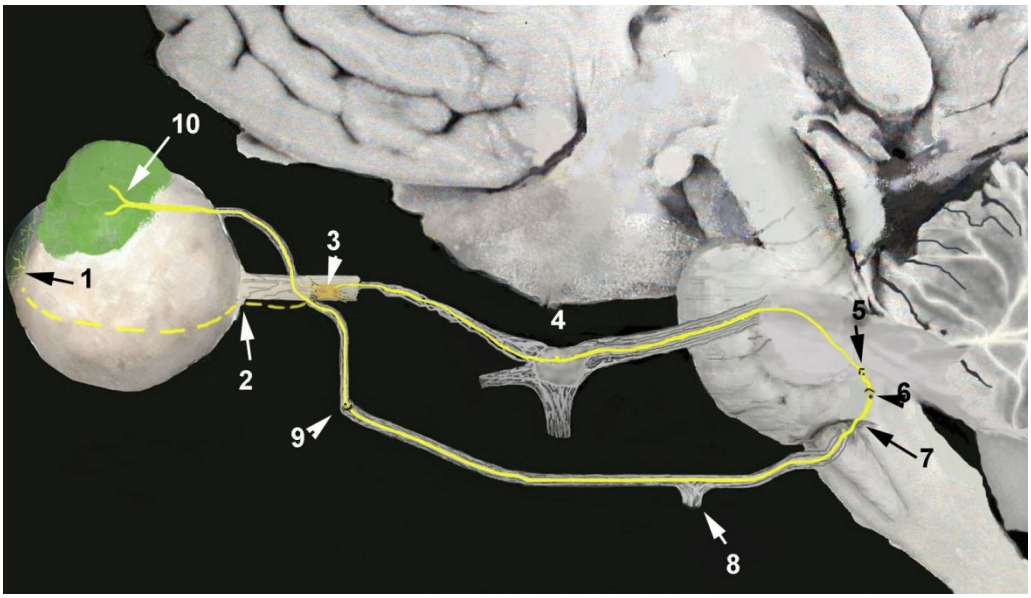

Figure 5: Neural pathway of the lacrimal functional unit; (1) sensory fibers in the cornea, (2) corneal sensory fibers traverse the long posterior ciliary nerve, (3) joining with nasociliary nerve, (4) trigeminal ganglion, (5) ipsilateral trigemnius tract, (6) lacrimal and salivatory nuclei, (7) effernt fibers merge into the seventh cranial nerve (facial nerve), (8) geniculate ganglion, (9) pterygopalatine nucleus, (10) innervation of the lacrimal gland

Subconscious stimulation of the nerve endings richly populating the cornea results in a generation of afferent nerve impulses through the ophthalmic branch of the fifth cranial nerve, the trigeminal nerve. These impulses travel through the trigeminal ganglion on to the mid brain (pons) where they synapse and the signal is integrated with cortical and other neural input. The efferent branch of the loop sends fibers through the pterygopalatine ganglion and further on to the main and accessory lacrimal glands (Stern, et al. 1998) (Stern, et al. 2004). A more detailed description of the neuronal pathway can be seen in Figure 5.

2.3 Lacrimal glands

The aqueous phase is mainly produced by the main and accessory lacrimal glands. The main lacrimal gland is located superior and lateral of each eye. The accessory lacrimal glands are small portions of lacrimal tissue incorporated in the upper and lower eyelids. The number of the accessory glands is approximately 50 for the glands of Krause (Figure 6), 5 for the glands of Wolfring and 1 for the caruncle, resulting in a total number of about 57 glands producing the aqueous phase. The tear film is transported to the lacriminal sac via ducts and is divided into aqueous portions in the upper and lower tear meniscus, the conjunctival sac and the pre-ocular tear film. The tear fluid is the transported towards the medial direction and leaves the ocular surface through the superior and inferior punctum and the lacrimal glands which finally merge into the nasolacrimal gland.

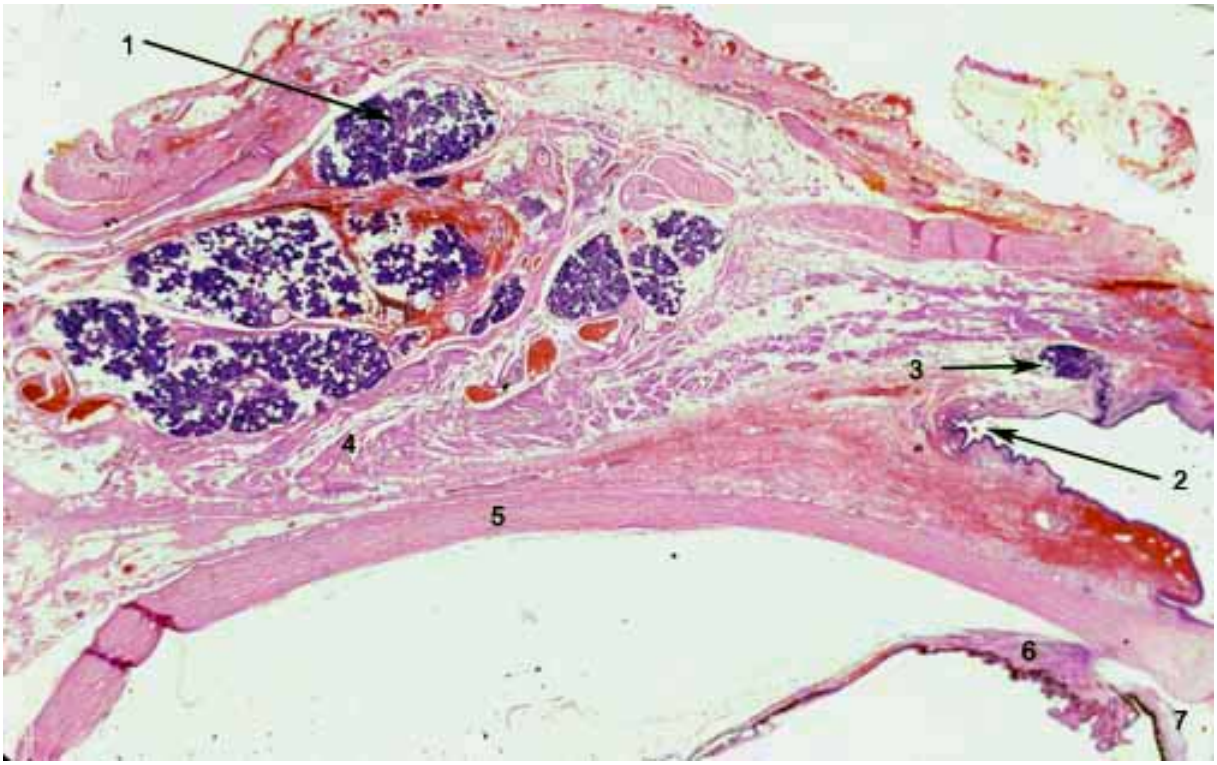

Figure 6: (1) lobus of the lacrimal gland; (2) fornix of the upper eyelid; (3) accessory lacrimal gland of Krause; (4) levator muscle; (5) sclera; (6) ciliary body; (7) iris; (http://www.images.missionforvisionusa.org/anatomy/2006/02/lacrimal-gland-human.html)

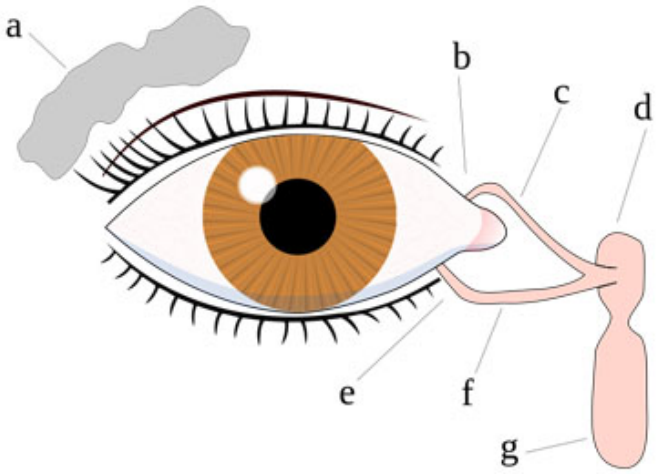

Figure 7: (a) lacrimal gland; (b) superior lacrimal punctum; (c) superior lacrimal canal; (d) lacrimal sac; (e) inferior lacrimal punctum; (f) inferior lacrimal canal; (g) nasolacrimal canal; http://en.wikipedia.org/wiki/Lacrimal_gland

2.4 Lipid layer

The thickness of the outermost layer of the pre-ocular tear film, the lipid layer, is estimated to be between 13 (J. P. Guillon 1998) and 100 nm (Norn 1979) (Bron, et al. 2004). The composition of the secreted meibomian lipids found in the tear film listed in

HC	WE	SE	Diester	TG	Alc	FFA	Polar	Reference
26-38	13-23	8-34	-	11-43	0.2	0-24	0.5	
-	35	30	8	4	2	2	16	
2	44	33	-	5	-	-	-	

Table 4.

HC	WE	SE	Diester	TG	Alc	FFA	Polar	Reference
26-38	13-23	8-34	-	11-43	0.2	0-24	0.5	(J. M. Tiffany 1978)
-	35	30	8	4	2	2	16	(Nicolaides, et al. 1981)
2	44	33	-	5	-	-	-	(Ohashi, Dogru und Tsubota 2006)

Table 4: HC: hydrocarbons; WE: wax esters; SE: sterol esters; TG: triglycerides; Alc: alcohol; FFA: free fatty acids; polar: polar lipids. [a] Recalculated percentages assuming all hydrocarbons are of environmental origin (Bron, et al. 2004)

The lipid layer fulfils a number of important functions for the tear film and thus also for the ocular surface. Aspects of the functionality of meibomian oil can be listed as follows:

In the lid margin reservoirs

- To maintain the lid skin in a hydrophobic state, and prevent tear overspill
- To resist contamination with sebum
- To prevent maceration of the lid skin by tears

On the tear film lipid layer

- Spread over the aqueous subphase, lower free energy and impart stability to the tear film
- To thicken the aqueous sub-phase (Marangoni effect)
- To retard evaporation
- To provide a smooth optical surface to the cornea
- To provide a barrier against foreign particles
- To provide some anti-microbial activity
- To seal the lid margins during prolonged closure (Bron, et al. 2004)

An abnormal constitution of the tear film lipid layer is often the reason for an improper behaviour of the tear film. Such a malfunction of the meibomian glands is often referred to as Meibomian Gland Dysfunctionality (MGD). It is estimated that about 75 % of DED cases

suffer from a lipid deficiency (Referenz Horwath-Winter, Referenz Heiligenhaus), whereas about 10 % suffer from an isolated alteration of the aqueous phase. It may thus be accepted that MGD is important, conceivably underestimated, and possibly the most frequent cause of DED due to increased evaporation of the aqueous tears (Knop, et al. 2011).

2.5 Production of the meibomian lipids

Meibomian lipids are produced in the meibomian glands located in the upper and lower tarsal plate (Figure 8). The number of separate glands in the upper lid varies between 25 and 40 with a median value of 31. The number of glands in the lower lid is given as approximately 20. The calculated total value of the upper glands is also higher: 26 µl compared to 13 µl in the lower tarsal plate.

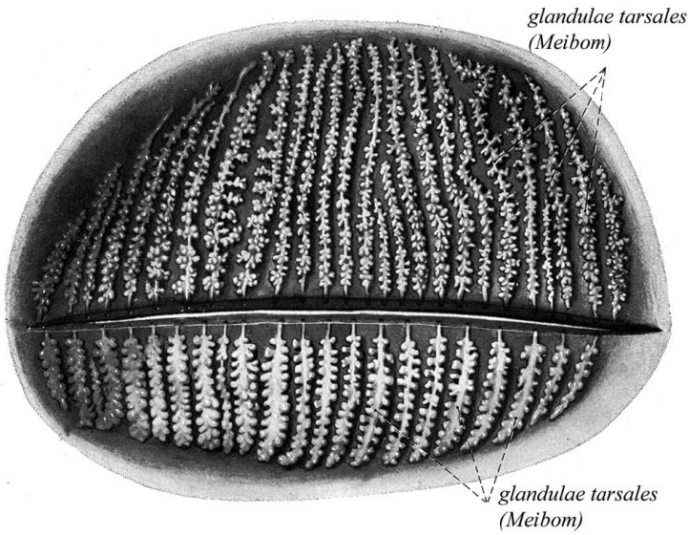

Figure 8: Inside view of the upper and the lower eyelid, with eyelids closed; Topography of the meibomian glands (Knop, et al. 2011)

The meibomian glands are composed of secretory acini that are connected via small ductules to the larger, long, straight central duct that extends throughout the length of the tarsal plate and opens onto the free lid margin close to the lid border (Figure 9A). The underlying transport mechanism are driving forces generated by (1) the pressure from the newly secreted meibomian lipids which are continuously forced into the ductules and the central duct, (2) the mechanical muscle activity of *m.orbicularis* and *m. Riolas* (Figure 9B).

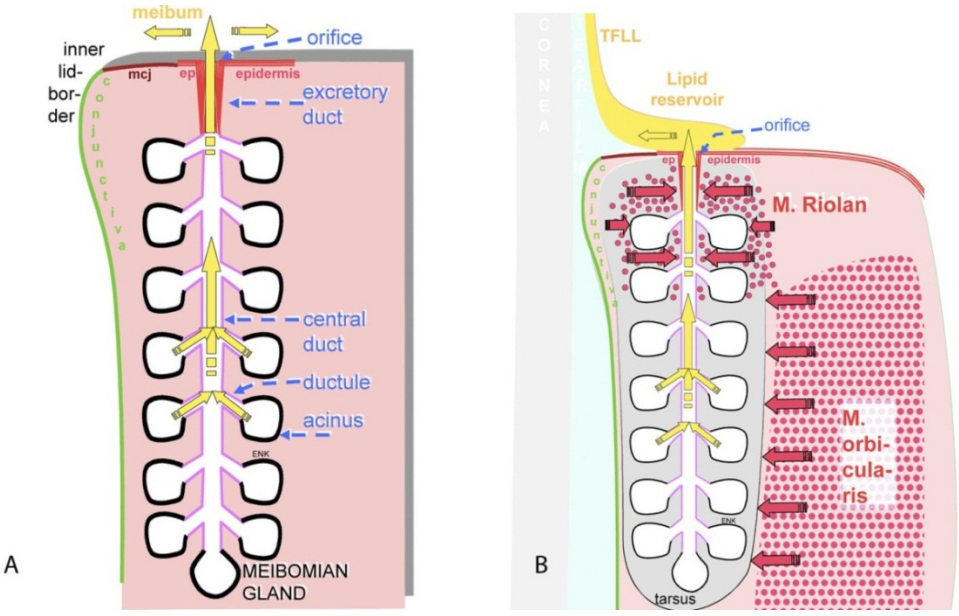

Figure 9: (A) Morphology of a single meibomian gland; ep: epidermis, mcj: mucocutaneous junction. The meibom lipids are prduced in the acini and transported through the system of ducts to the posterior part of the lid margin (indicated with yellow arrows); (B)

3 Risk factors for DED

Popular risk factors which harm the construct of the tear film include contact lens wearing (C. G. Begley, et al. 2000), working in air conditioned environment (Doughty, Blades und Ibrahim 2002), visual display terminal usage (Nakamura, et al. 2010) (Uchino, et al. 2008)and the hormonal status (Schaumberg, et al. 2001). Additionally, Laser Assisted in Situ Keratomileusis (LASIK) surgeries (Toda, et al. 2001) and autoimmune disorders like Sjogrens Syndrome (Nakamura, Kawakami und Eguchi 2006) are contributing to the destabilization process. This chapter describes the underlying processes of these risk factors.

3.1 Contact Lens Wearing

It has been reported previously that the wearing of contact lenses is often accompanied by DED symptoms. Several studies reported that 50% to 75% of contact lens wearers had subjective symptoms of ocular discomfort ((C. G. Begley, et al. 2000) (Nichols, et al. 2005) (Brennan und Efron 1989). With the aforementioned lower estimate of 50% , approximately 17 million Americans are estimated to suffer from contact lens related DED.

With the contact lens put in the pre-ocular tear film separates into a pre- and a post-lens tear film. The proposed underlying mechanisms for the high rate of manifestation of DED

within contact lens wearers are different and suggest the presumption that the influence of contact lenses on the tear film is multifactorial.

Nichols and Sinnot showed that several factors such as female gender, lenses with higher nominal water content, a high tear evaporation rate and increased tear film osmolarity was reported.

An explanation for the hyperevaporation of the tear film may be the reported decline of meibomian glands in the tarsal plates. Since a thining of the pre-lens tear film goes hand in hand with increasing shear forces between the contact lens surface and the inward surface of the tarsal plates.

In addition the shear forces between the ocular surface epithelia and the inward surface of the contact lens lead to a lowering of the sensory ability of the cornea (Gilbard, Gray und Rossi 1986). As mentioned in *2.2.6 Lacrimal functional unit* the afferent input of the cornea is an important input for the neuronal regulation loop of the lacrimal glands.

The resulting increased osmolarity is then often the reason for an inflammation of the ocular surface. In addition, hyperosmolarity causes ocular surface cell damage, which can be visualized by ocular surface staining. This damage occurs because ocular surface cell membranes are permeable; when they are exposed to hyperosmotic tears, water flows out of the cells in an attempt to balance the osmolarity of the intracellular fluid with the osmolarity of the surrounding tears. When this happens, ocular surface cells can become dehydrated, which damages cell membranes and changes the way proteins protect the ocular surface (Foulks 2009).

Thai et al additionally reported that a lacking biocompatibility of the contact lens surface could lead to an improper lubrication and distribution of tear fluid on the contact lens (Thai, Tomlinson und Simmons 2002).

3.2 Visual display terminal usage

The significant higher prevalence of DED within visual terminal users compared to other workers has been estimated to be approximately 10% in male and 21% in female users (Uchino, et al. 2008). The reason for the higher risk is proposed as to be the significant lower blink rate during visual display tasks, especially during tasks with a high mental load (Tsubota

und Nakamori, Dry eyes and video display terminals. 1993). Nakamura et al has additionally shown that the total secretion as determined with Schirmer test (without anaesthesia) and the number of acinar cell in the lacrimal gland significantly decreased during a prolonged visual display terminal usage over twelve years and more than eight hours per day. The characteristic features of morphological change in the LG were enlargement of acinar cells accompanied by filling with an increased volume of secretory vesicles and loss of intracellular cell structure. They propose that a suppressed stimulation of the corneal surface due to a chronic reduced blink rate may be the reason for this mechanism.

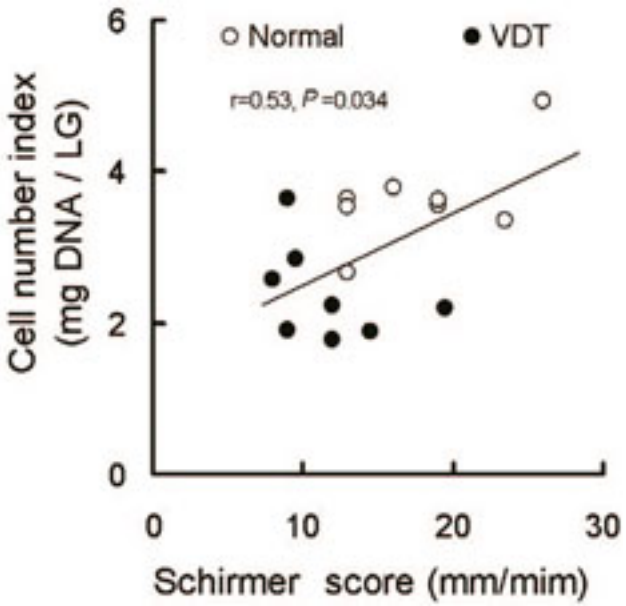

Figure 10: Correlation between tear production and LG cell number. Pearsons correlation coefficient testing was r = 0.53 (P = 0.034, n = 16), (Nakamura, et al. 2010)

3.3 Hormonal Status

It is well known that women are significantly higher affected by DED than men, especially after the menopause. The reason for this has been proposed as the effect of postmenopausal hormone therapy. A large scaled study showed an approximately 70% increased risk of the women, using estrogen alone, and a 30% higher risk in women who used estrogen in combination with progesterone or progestins (Schaumberg, et al. 2001). These data suggest that progesterone/progestine therapy has seems to have a protective effect with regard to the manifestation of DED: Since there is a strong connection with sexual hormones and sebaceous glands, the biologically most valid explanation is a relationship of postmenopausal hormone replacement therapy and the meibomian glands, resulting in the evaporative form of DED (Schaumberg, et al. 2011). In addition, also

androgens have a significant effect on the secretion capacity of meibomian glands. In a way that they observed 1) a significant increase in the frequency of appearance of tear film debris, an abnormal tear film meniscus, irregular posterior lid margins, conjunctival tarsal injection, and orifice metaplasia of themeibomian glands; 2) a significant increase in the degree of ocular surface vital dye staining; 3) a significant decrease in the tear film breakup time and quality of meibomian gland secretions; and 4) a significant increase in the frequency of light sensitivity, painful eyes, and blurred vision. In addition, the use of antiandrogen pharmaceuticals was associated with significant changes in the relative amounts of lipids in meibomian gland secretions (Krenzer, et al. 2000).

3.4 Laser Assisted in Situ Keratomileusis (LASIK) surgeries

To investigate the relationship between LASIK surgeries and the manifestation of DED, Toda et al conducted a retrospective, interventional case series. They included 124 eyes of 64 consecutive patients and examined for a dry eye symptom, Schirmer test with anesthesia, tear clearance rate, tear break-up time, vital staining for ocular surface, corneal sensitivity, and blink rate. All values were compared before and after surgery (1 month, 3 months, 6 months, and 1 year).

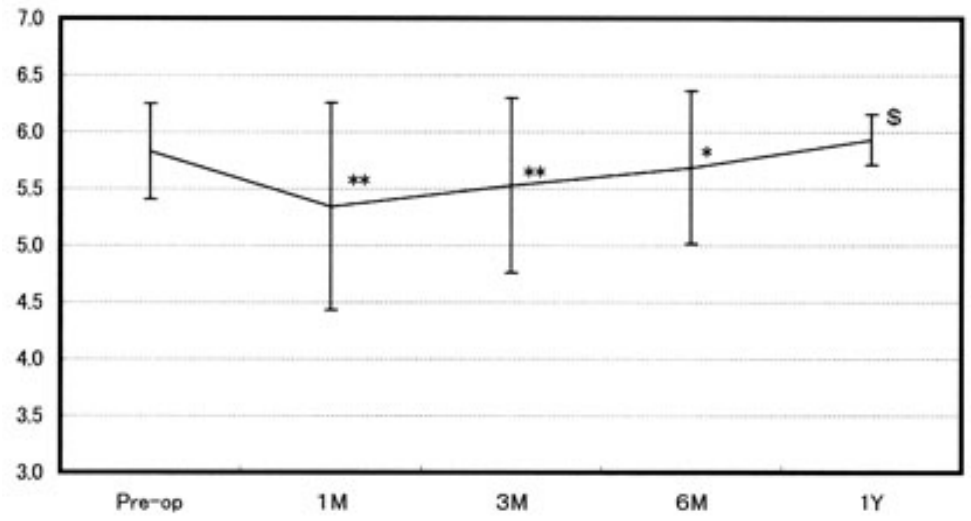

Figure 11: Corneal sensitivity before and after laser assisted in situ keratomileusis ; *P < 0.05; **P < 0.01 (decreasing); $P < 0.01 (increasing) (Toda, et al. 2001)

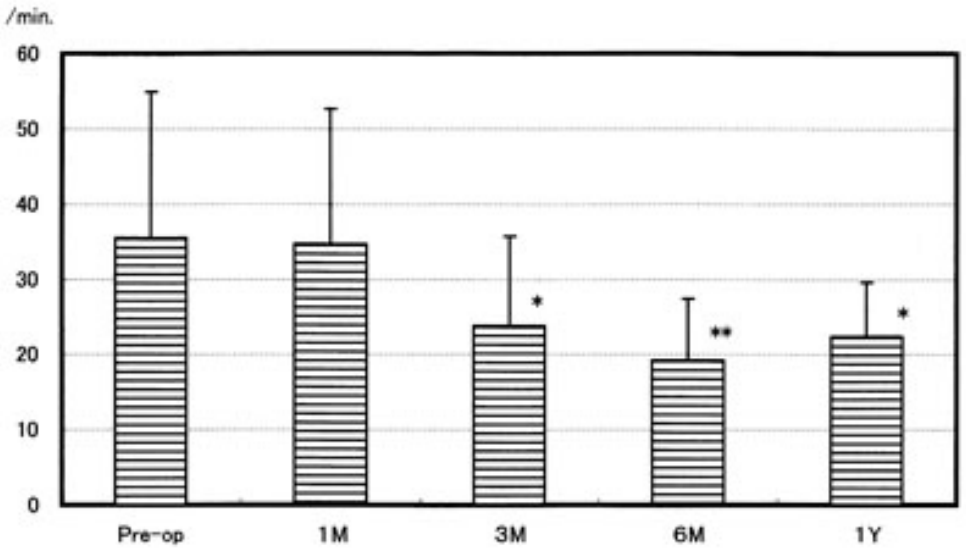

Figure 12: Blink rate before and after lases assisted keratomileusis (*P < 0.05; **P < 0.01); (Toda, et al. 2001)

3.5 Sjogren Syndrome

Sjögren Syndrome is an autoimmune disorder that affects exocrine glands, including salivary and lacrimal glands. The incidence rate of this disease is estimated to be approximately 3.9 per 100.000, whereas women have a significant higher incidence (6.9 / 100.000) compared to 0.5/100.000 within men (Schaumberg, et al. 2011). The proposed mechanism is an infiltration of mononuclear lymphoid cells of the gland tissue, thereby replacing the secretory cells. The infiltration consists mainly of T-cells, but also macrophages and plasma cells can be found.

The focal infiltration consists mainly of T cells, but also macrophages and plasma cells. Normally, lymphocytes circulate in the blood and invade the tissue as a response to infection or injury. This is a complex process regulated by a range of adhesion molecules on the inflammatory cell surfaces and the endothelial cells. The lymphocytes adhere to the endothelium by means of adhesion molecules and can move from circulation to tissue. Interestingly, serum levels of an adhesion molecule related to epithelial cells, E-cadherin, has been found to be increased in Sjögren's syndrome indicating the close interaction between epithelial cells and lymphocytic organization (Jonsson, et al. 2011).

In addition to the reduced functionality of the lacrimal glands and the decreasing amount of aqueous solution, also the meibomian gland drop out seem to be affected by this constitution. Shimazaki et al reported a significant higher incidence of meibomian gland obstruction within patients suffering from Sjögren Syndrome (38.9 % in DED patients with

Sjögren Syndrome compared to 11.1 % in DED patients without Sjögren Syndrome) (Shimazaki, et al. 1998). In accordance with these results Goto et al showed a higher evaporation rate in DED with Sjogren Syndrome compared to normal DED patients (Goto, Matsumoto, et al. 2007).

3.6 Aging

It has been shown that the aging process also affects the morphology of the meibomian glands. Den et al therefore conducted a study within 354 eyes were investigated for abnormalities of the lid margins, the meibomian glands, the ocular surface epithelium and tear function (Den, et al. 2006). Only few patients below an age of 50 showed alterations in the observed parameters whereas such abnormalities occurred significantly more often in the elderly population of subjects. Hykin and Bron have reported in a cross-sectional study with 80 subjects between 5 and 87 years old that an increase in eyelid margin vascularity, keratinization, telangiesctasia, and opacity of meibomian gland secretions was observed with aging (Hykin und Bron 1992). Sullivan additionally also showed significant alterations in older versus younger individuals' polar and neutral lipid profile derived from meibomian gland secretions (Schaumberg, et al. 2011) (Sullivan, et al. 2002)

4 Diagnostic Procedures

This chapter describes both the common diagnostic clinical procedures and innovative techniques, used mainly for scientific purposes.

Due to the diversity of risk factors and etiology as well as the complex interactions between the layers, the precise diagnosis of DED is challenging. A number of standard assessment procedures for tear film disorders have been developed and can be divided into subgroups: questionnaires to assess the subjectively reported symptoms; investigations of ocular surface damage via staining; the demonstration of the tear film instability by Tear Film Break Up Time as determined with Fluorescein (TFBUT); and the lacrimal gland production capacity through the Schirmer test. It is remarkable that these clinical investigations correlate quite poorly with the subjectively reported symptoms and vice versa (Begley, Chalmers, et al. 2003). This can be explained by the highly dynamical characteristics of the tear film and its sensitivity to the surrounding environment as well as to the often invasively performed diagnostic procedures.

4.1 Invasive Tear Film Break Up Time (TBUT)

The parameter acquired for this test is the time following a blink until a significant dry out area is detected on the corneal surface. Immediately after a blink, the tear film ideally distributes homogeneously over the ocular surface and remains stable until the next blink action occurs. Especially, in DED patients, an early break up after a blink action indicates instability of the tear film. The most common way to determine the break up time is the investigation of the tear film after the instillation of the dye fluorescein through a yellow filter (Figure 13). Dry areas are thereby detected as dark areas on the corneal surface. Ophthalmologists normally take three consecutive measurements of the break up time and calculate the mean value.

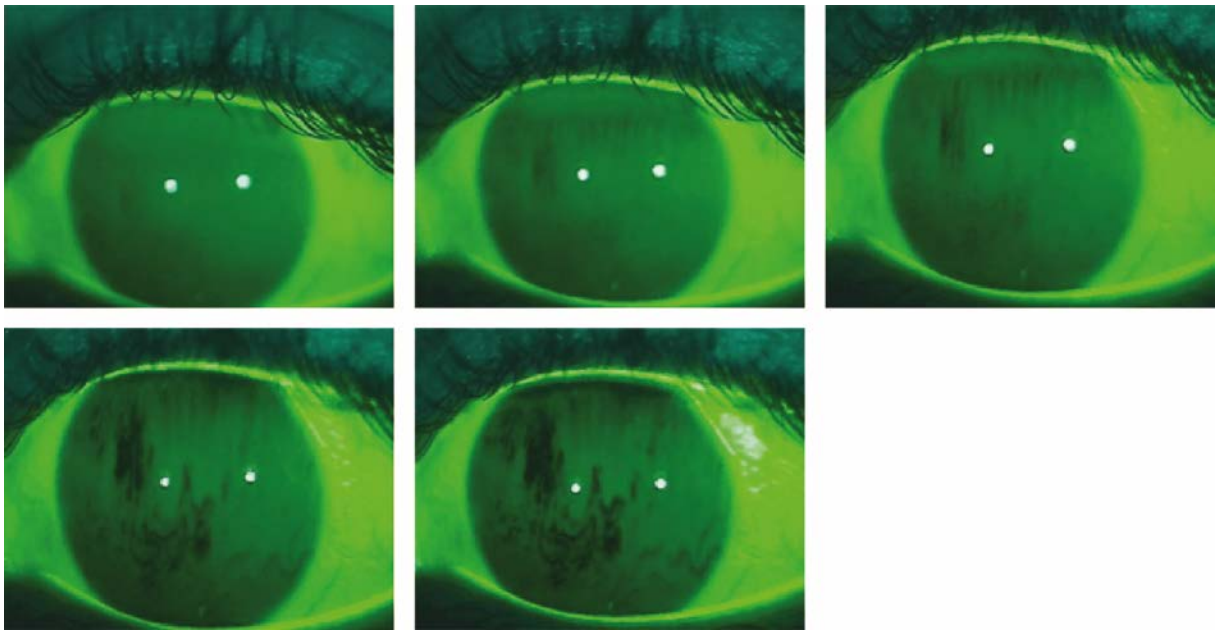

Figure 13: Tear film break up as investigated with fluorescein staining; starting with a homogeneous distribution of the tear film (upper left) to a large area break up after approximately 10 seconds (lower right); http://users.cecs.anu.edu.au/~tamir/aovsm/index.html

A suitable cut-off value for the tear film break up time seems to be 10 seconds, resulting in a sensitivity of 72% and a specificity of 62%. The cut off value is strongly dependent on the amount of fluorescein instillation. Historically, the technique for evaluating TFBUT has lacked consistency. Large and varying amounts of sodium fluorescein (up to 50 µl) were used; times were determined by counting aloud and using less sophisticated instrumentation. Such techniques yield varying results (Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). 2007).

4.2 Schirmer Test (without anaesthesia)

The principle of the Schirmer test is to hang a filter tip into the lower lid margin for five minutes. The amount in the filter tip is then though to be proportional to the quantity of the tear fluid. Due to this fact, the Schirmer test (5x35mm Whatman No 1) is mainly conducted in order to diagnose aqueous deficient DED.

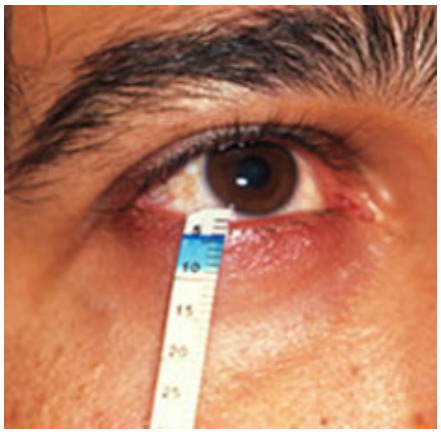

Figure 14: Filter tip is put slightly lateral in the lower eyelid margin (http://www.apotheken-umschau.de/diagnose/schirmer-test)

A proposed cut off value of 5.5 mm results in a specificity of 83% and a sensitivity of 85%. Although the Schirmer test without anaesthesia is an easy way to make assumptions on the quantity of the tear fluid it still has some drawbacks. Clinch et al have therefore investigated the kinetic parameters of the fluid within the filter strip. They investigated 50 patients and discovered a strong non-linear wetting of the filter tip due to an initial causation of a corneal sensation, which induces reflexive tearing. These results indicate that the Schirmer test measures not solely the amount of basic tearing.

4.3 Osmolarity measurement

More recently, devices for measuring the tear film osmolarity have also been introduced, e.g. Tearlab® (Tearlab®, Osmolarity System, Ocusense, San Diego, CA, United States).

The principle is that certain salts are dissolved in the aqueous tear fluid. Since dry eye occurs either due to a high evaporation or a lack of quantity of the tear fluid but the amount of salts remains stable, the concentration and thus the osmolarity increases significantly within DED patients. A chronically high osmolarity of the tear fluid could potentially lead to a chronic inflammation of the corneal surface tissue. .This damage occurs because ocular surface cell membranes are permeable; when they are exposed to hyperosmotic tears, water flows out of the cells in an attempt to balance the osmolarity of the intracellular fluid with the osmolarity of the surrounding tears. When this happens, ocular surface cells can become dehydrated, which damages cell membranes and changes the way proteins protect the ocular surface (Foulks 2009).

Figure 15: Tearlab for osmolarity measurements; left: TearLab device; right: sensor (http://thedryeyereview.com/2011/02/tearlab-osmolarity-%E2%80%93-a-new-gold-standard-validated/)

A proposed specificity and sensitivity for this parameter was presented by Sullivan (B. Sullivan 2004). A concentration of greater than 318mOsm was proposed for the diagnosis of DED, thereby resulting in a specificity of 84% and a sensitivity of 94%. This method is still discussed controversially. Kanal et al suggested taking the average of three consecutive measurements to reduce the variability of this test (Khanal und Millar 2011). The sensitivity and specificity of the osmolarity measurement are still under discussion. Messmer et al conducted a study, within they investigated 200 persons, recurited from both the healthy and from the DED population. They used artificial tear instillation in order to demonstrate the effect of the osmolarity measurement. Measurement were conducted with the Tearlab Osmolarity measurement system and were not found to correlate with the use of artificial tears. Technical problems with the Tearlab, reflex tearing, or the difficulty in establishing a dry eye diagnosis with the recommended tests may account for these results.

4.4 Staining of the ocular surface

One possible way to evaluate the damage of the corneal surface cells is the staining with specific dye and the investigation of the dye through proper filters. Mainly three days have been established to suit this purpose: fluorescein, lissamine and rose bengal (Figure 16). Sodium fluorescein permeates into the intercellular space associated with any epithelial cellular disruption. Fluorescence should be observed with a blue excitation filter over the white light source and can be highlighted by placing a Wratten #12 yellow filter over the slit-lamp objective. Fluorescein is highly tolerable and a single application does not cause stinging. A disadvantage to using fluorescein is that the fluorescent tear film may obscure

corneal staining measurements. To avoid inaccurate staining measurements, conduct tear film assessments (tear film break up time and tear film meniscus height) first, allowing time for the fluorescein to diffuse from the tear film to the cornea, then measure corneal staining (Ramsey 2011).

Lissamine green is an acidic, synthetically produced, organic dye that has been historically used in food products. Since the introduction of lissamine green, clinical reports have indicated its use as a stain to diagnose ocular surface disease. Lissamine green stains dead and degenerate cells, yet does not stain healthy epithelial cells. The literature contains no reports of toxicity, and, at 1% concentration, this dye is not associated with stinging or discomfort. Studies have shown that lissamine green and rose bengal have similar staining profiles, yet lissamine green is better tolerated by patients (Ramsey 2011).

Rose bengal stains dead and devitalized cells, as well as mucus, and should be observed using a white light source. Mucins and other tear film components block rose bengal staining, thus a break in the tear film must be present for rose bengal to penetrate the ocular surface. Prior to the introduction of lissamine green in 1973, rose bengal was the preferred dye for assessing conjunctival staining. However, it is widely known that rose bengal causes stinging—sometimes severe—in most patients and is mildly toxic to the ocular surface. In addition, rose bengal stains healthy epithelial cells and, therefore, is not technically considered a “vital” dye. Based on its shortcomings, rose bengal has been losing its popularity in clinical practice (Ramsey 2011).

A possible way of evaluating the staining is the Oxfors staining scheme as described by Bron et al (Bron, Evans und Smith 2003) , Figure 17.

Figure 16: Staining of ocular surface with: fluorescein green (upper left); lissamine green (upper right) and rose bengal (lower left)

PANEL	Grade	Criteria
A	0	Equal to or less than panel A
B	I	Equal to or less than panel B, greater than A
C	II	Equal to or less than panel C, greater than B
D	III	Equal to or less than panel D, greater than C
E	IV	Equal to or less than panel E, greater than D
>E	V	Greater than panel E

Figure 17: Oxford staining schema as described by Bron et al (Bron, Evans und Smith 2003)

4.5 Tear Film Meniscus Radius and Height

The principle of this method is the determination of the geometrical features of the tear menisci, especially of the lower tear film meniscus. Both the radius and the tear meniscus height are therefore considered to be suitable parameters. It has been shown that these parameters correlate with the quantity of the tear fluid as determined with the Schirmer test 4.2.

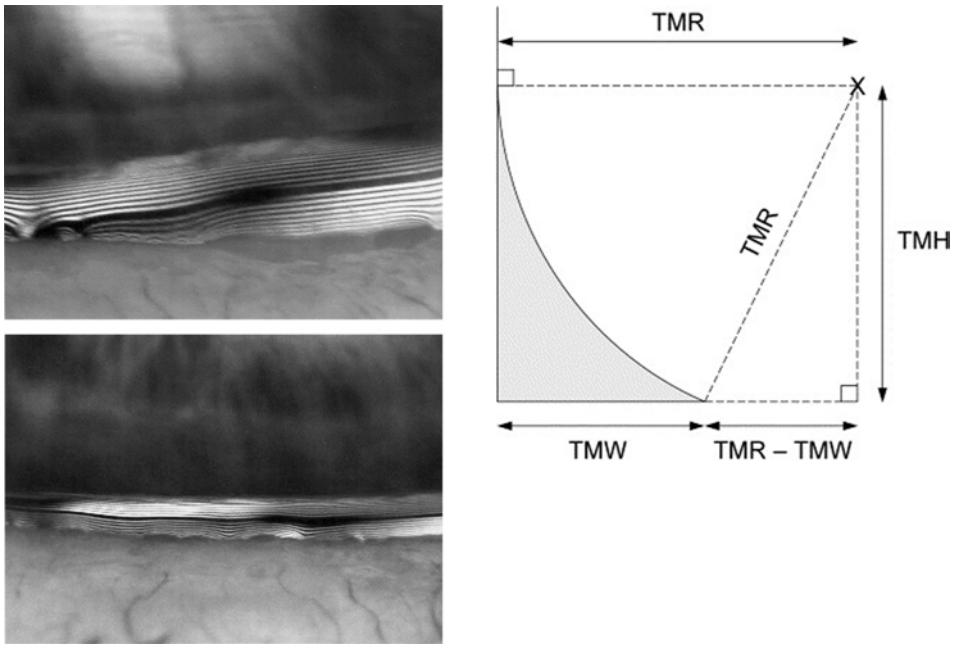

Figure 18: (left) Projection of black and white stripes on the lower tear film meniscus; (right) Geometrical features of the tear film meniscus: TMR…Tear meniscus radius; TMH…Tear meniscus height; TMW…Tear meniscus width

A possible way for the determination of the desired geometrical features was proposed by Oguz et al (Oguz, Yokoi und Kinoshita 2000), **Fehler! Verweisquelle konnte nicht gefunden werden.**. The subject is placed in front of a special slit lamp system; a series of black and white stripes (black and white; each 4mm wide) are projected onto the lower tear film meniscus; the images are digitally stored on a computer; images are transferred to a computer and image analysis software used to calculate the radius of curvature of the meniscus by applying the concave mirror formula.

Images of the lower tear film meniscus can also be acquired through the usage of corneal confocal microscopy. The principle of this technique is shown in Figure 19.

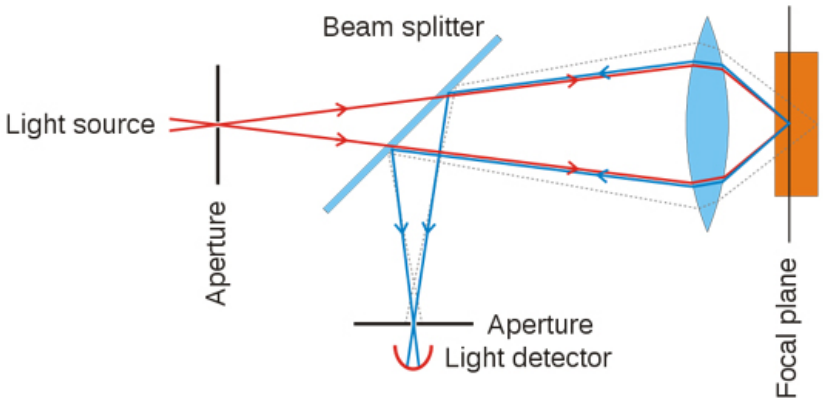

Figure 19: Functional principle of Confocal Microscopy: a laser passes through an aperture and a beam splitter; the rays are then focused on a plane through an objective lens onto the sample; the reflected laser light is then recollected by the objective and brought to the light detector via a beam splitter. The desired depth of the focus plane is controlled by the aperture just before the light detector; a great proportion of the light is thereby blocked.

A resulting image sequence is shown in Figure 20. Parameter can easily be extracted manually within image processing software.

Different thresholds, sensitivity and specificity parameters have been published. Mainstone et al chose a threshold of <0.35 to diagnose DED, resulting in a sensitivity of 93.3 % and a specificity of 67% (Mainstone, Bruce und Golding 1996). In contrast, Farrell et al reported a sensitivity of 73% and a specificity of 67 % at a diagnostic threshold of 0.18 mm (Farrell, et al. 2003). It has also been reported that the height of the lower tear film meniscus significantly increases after plugging the nasolacrimal ducts with punctual plugs.

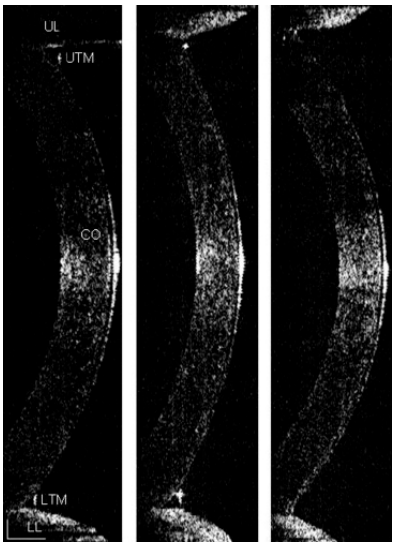

Figure 20: Image series of the corneal surface recorded through confocal microscopy; UL… upper lid, LL… lower lid, UTM… upper tear meniscus, LTM… lower tear meniscus.

4.6 Non Invasive Tear Film Break Up Time (NITBUT)

Another way to determine the overall tear film stability is to determine the non-invasive tear film break up time. Mengher et al were among the first to describe the dynamic distortion of a pattern projected on the ocular surface as a result of the local drying out of the tear film (Mengher, et al. 1985). Meanwhile several devices have been developed in order to fulfil this purpose. Guillon et al proposed a device called TearScope (Tearscope, Keeler, Broomall, Pennsylvania, Figure 21). Often videokeratographs, intended to be used for the investigation of discontinuities of the corneal surface, are also used to extract the non-invasive parameter. Any ruptures of the projected placido rings thereby related to the tear film stability (Bruce, Mainstone und Golding 2001) Figure 22.

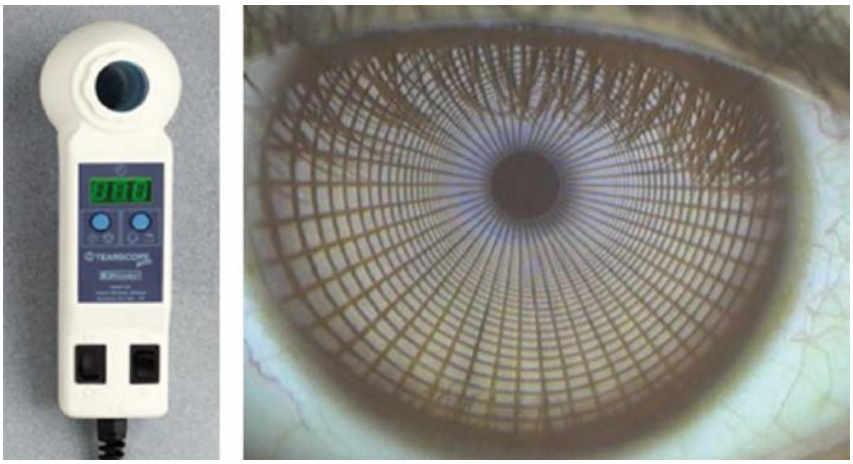

Figure 21: Principle of the TearScope Plus (Tearscope, Keeler, Broomall, Pennsylvania)

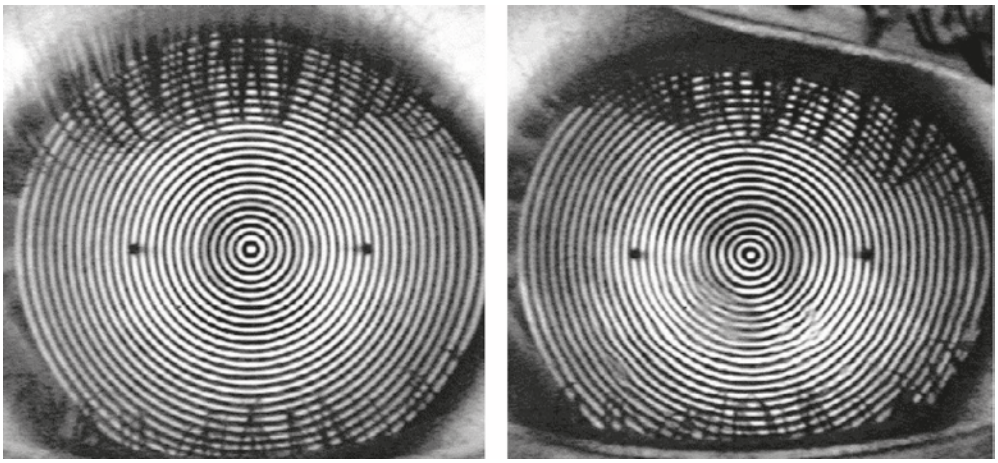

Figure 22: Tear film break up as investigated with a videokeratograph (TMS-1™, Computed Anatomy Incorporated, New York)

The parameter of the non- invasive tear film break up time is still discussed controversially. Guillon et al reported that within a large scale study of contact lens wearers in 35 % of the

cases, the tear film break up could not be observed before the next blink occurred (Guillon, et al. 1997). A cut-off value of 10 seconds or lower results in a specificity of 85% and a sensitivity of 83% (Mengher, et al. 1985).

4.7 Non Invasive Tear Film Lipid Layer Stabilization Time

The background of the kinetics of the tear film lipid layer spread after a blink action has been reviewed by Bron et al (Bron, et al. 2004) and Butovich (Butovich 2011) . With the upward movement the superior tarsal plate draws lipids from the region between the apposed lids. This leads to a rapidly upward propagating lipid layer over the underlying aqueous layer which finally decays to a stabilization of the tear film lipid layer. It is supposed that initially the polar lipids spread rapidly over the aqueous layer, followed by a retarded movement of the non-polar lipids (Yokoi, Yamada, et al. 2008). Owens and Phillips described the decay of the lipid spread velocity by a logarithmic function (Owens und Phillips 2001). They investigated the tear film lipid layer spread through the observation of particle movement in the tear film of normal adult subjects. The lipid layer movement has been shown to stabilize after approximately one second, which is comparable to the uncorrected lipid layer stabilization time acquired by our device within Subgroup 1(mild). Yokoi et al used a video interferometer (DR-1, Kowa, Tokyo, Japan) for this purpose, and proposed a linear viscoelastic model as a theoretical attempt to describe the tear film lipid spread (Yokoi, Yamada, et al. 2008).

Goto and Tseng have shown that the spreading time of the lipid layer after a blink is significantly prolonged in patients with DED compared to healthy subjects (Goto und Tseng, Differentiation of lipid tear deficiency dry eye by kinetic analysis of tear interference images. 2003) . These findings were extended by a second study on the lipid layer behavior, which investigated the dynamics of this outermost layer before and after a punctual occlusion (Goto und Tseng, Kinetic analysis of tear interference images in aqueous tear deficiency dry eye before and after punctal occlusion. 2003). These studies reported that the lipid layer stabilization time was significantly reduced after this therapeutic procedure, indicating that the performance of the lipid layer is strongly influenced by the underlying aqueous tear fluid. Additionally, the lipid layer spreads more uniformly immediately after a blink in healthy eyes. Recording were taken with the DR-1 interferometer (DR-1, Kowa, Tokyo, Japan).

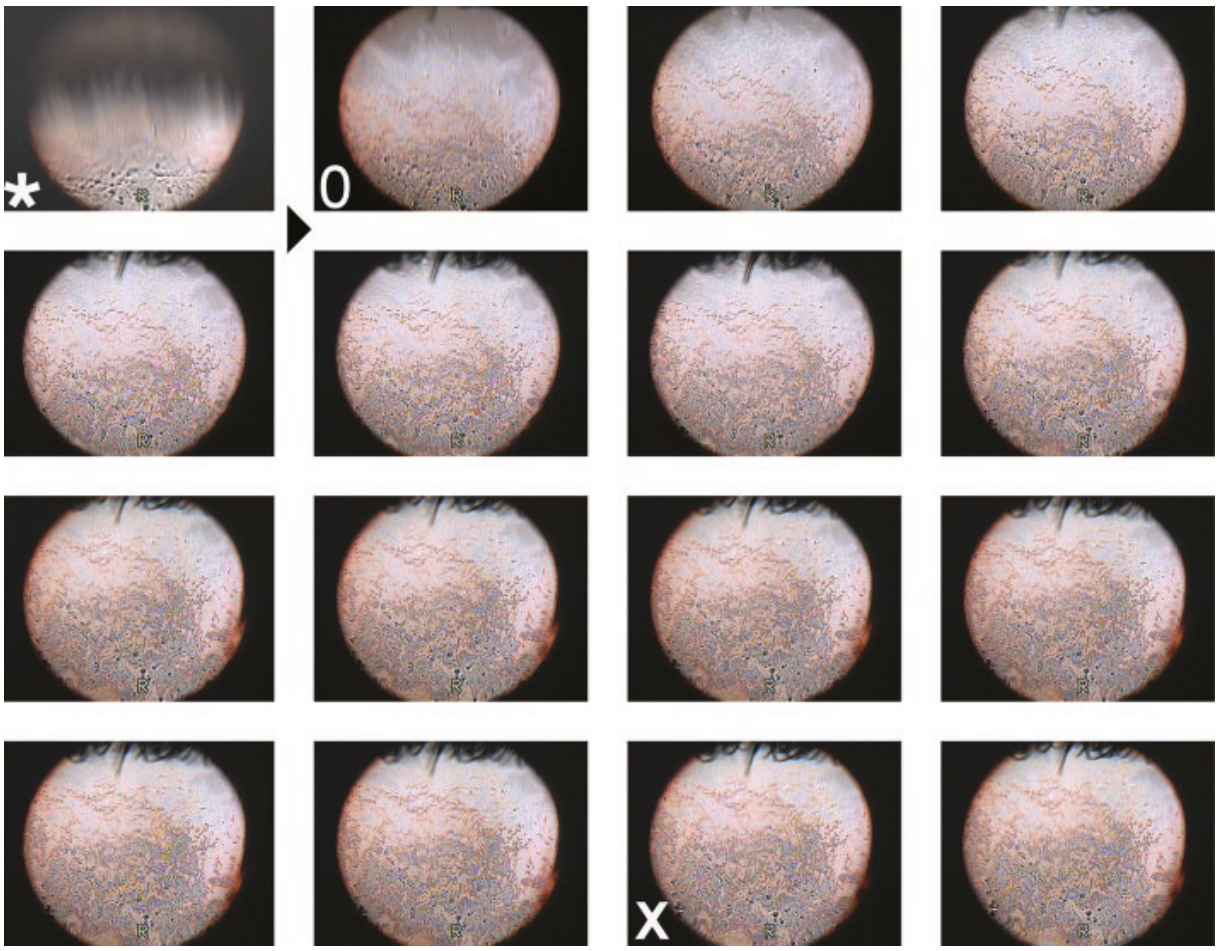

Figure 23: Representative sequential images from a patient with aqeuous deficient DED (Goto und Tseng, Kinetic analysis of tear interference images in aqueous tear deficiency dry eye before and after punctal occlusion. 2003)

4.8 Meibometry

This technique was developed in order to quantify the amount of lipids of the pre ocular tear film layer. It was first described by Chew et al. The principle is to place a pre formed loop of meibometry tape, mount it on a Goldman applanation tonometer and (Keeler, Windsor, England). With the subject looking upward without blinking, the lower lid is gently averted, and the loop is pressed onto the central third of the lid margin with sufficient pressure to obtain an imprint across the width of the tape, without bending the handle of the loop. Contact is maintained for 3 seconds. After blotting, the tape is kept in air for 3 minutes to allow evaporation of any tears picked up from the lid. The loop is placed in the reading head of the Meibometer (e.g. MB 550; Courage & Khazaka Electronic GmbH, Cologne, Germany). tape is then scanned across the reading window of the photosensor of the Meibometer, and

the maximum reading is taken from the densest part of the blot as it passes the line of the window.

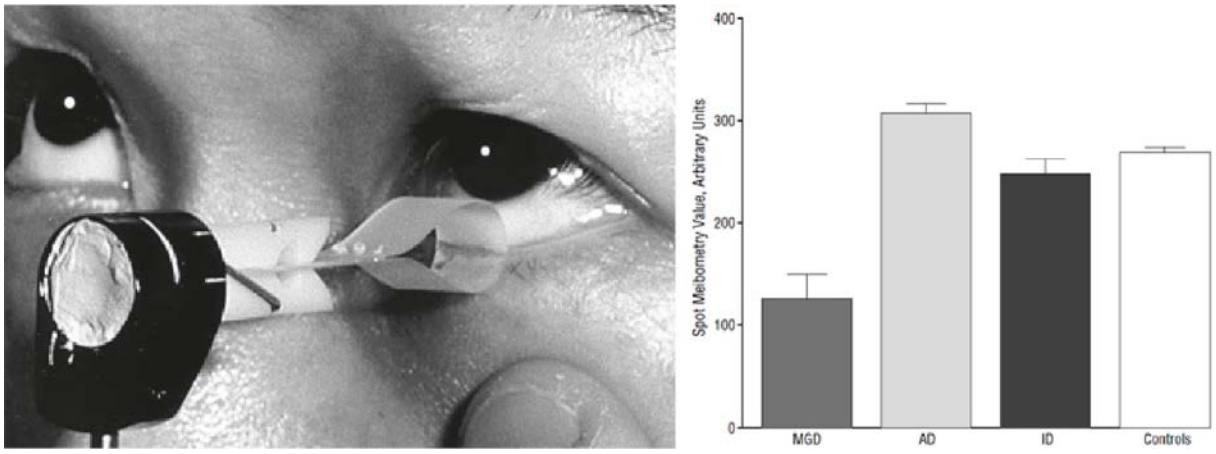

Figure 24: (left) A view of the system for taking oil imprints from the lid margin (meibometry); (right) Comparison of lipid quantity for different groups: MGD … meibomian gland dysfunction, AD … aqueous deficient DED, ID …incomplete DED

Yokoi et al conducted a study within 42 patients with clinically diagnosed dry eye, classified as 12 patients with MGD, 10 with aqueous tear deficienty, 2 with combined MGD and aqueous deficient DED, 12 with incomplete DED (TFBUT <=5; Staining score >=2, Schirmer test okay) and 6 non DED patients, were compared with 41 healthy control subjects. The result can be seen in Figure 24.

Another way to evaluate the meibomian gland dropout was proposed by Yokoi et al (Yokoi, Komuro, et al. 2007). The meibomian glands are thereby illuminated with infrared diodes; an infrared sensitive CCD chip then acquires images which are digitally saved on a computer.

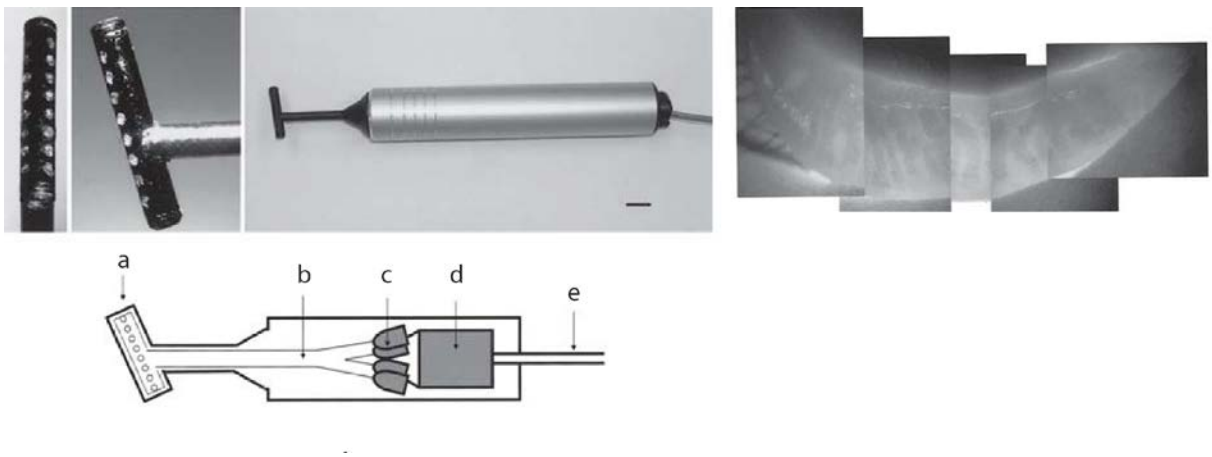

Figure 25: Top: The probe head; Bottom: Schematic diagram of the internal structure of the probe. a, windows through which the target area is illuminated; b, optical fibers; c, infrared light-emitting diodes; d, electrical circuit; e, cord. Right: Image sequence of meibomian glands of the lower eyelids (Yokoi, Komuro, et al. 2007)

5 LipidViewer

The intended use of the LipidViewer is to make recordings of the dynamic processes of the ocular surface, especially following a blink and thereby following a regeneration of the tear fluid. A detailed description of the intended use of the lipid viewer can be found in chapter 6.4. With this device two different investigations can be performed:

- the tear film stability through the non-invasive tear film break up time (Figure 26, Chapter 4.6),
- the lipid layer stabilization time (Figure 27, Chapter 4.7).

The system consists of the following parts, which are described in detail in the following chapters:

- the illumination unit,
- the control unit,
- the camera,
- the insulating transformer,
- the laptop.

The subject is placed in front of the device, with the skin around the eye coming in brief contact with the illumination unit. It is important to change the foil for one time usage due to hygienic reasons (Figure 28 A,B). After the patient data is put in, the recording starts with the non-invasive tear film break up time, followed by the recording for the lipid layer stabilization time.

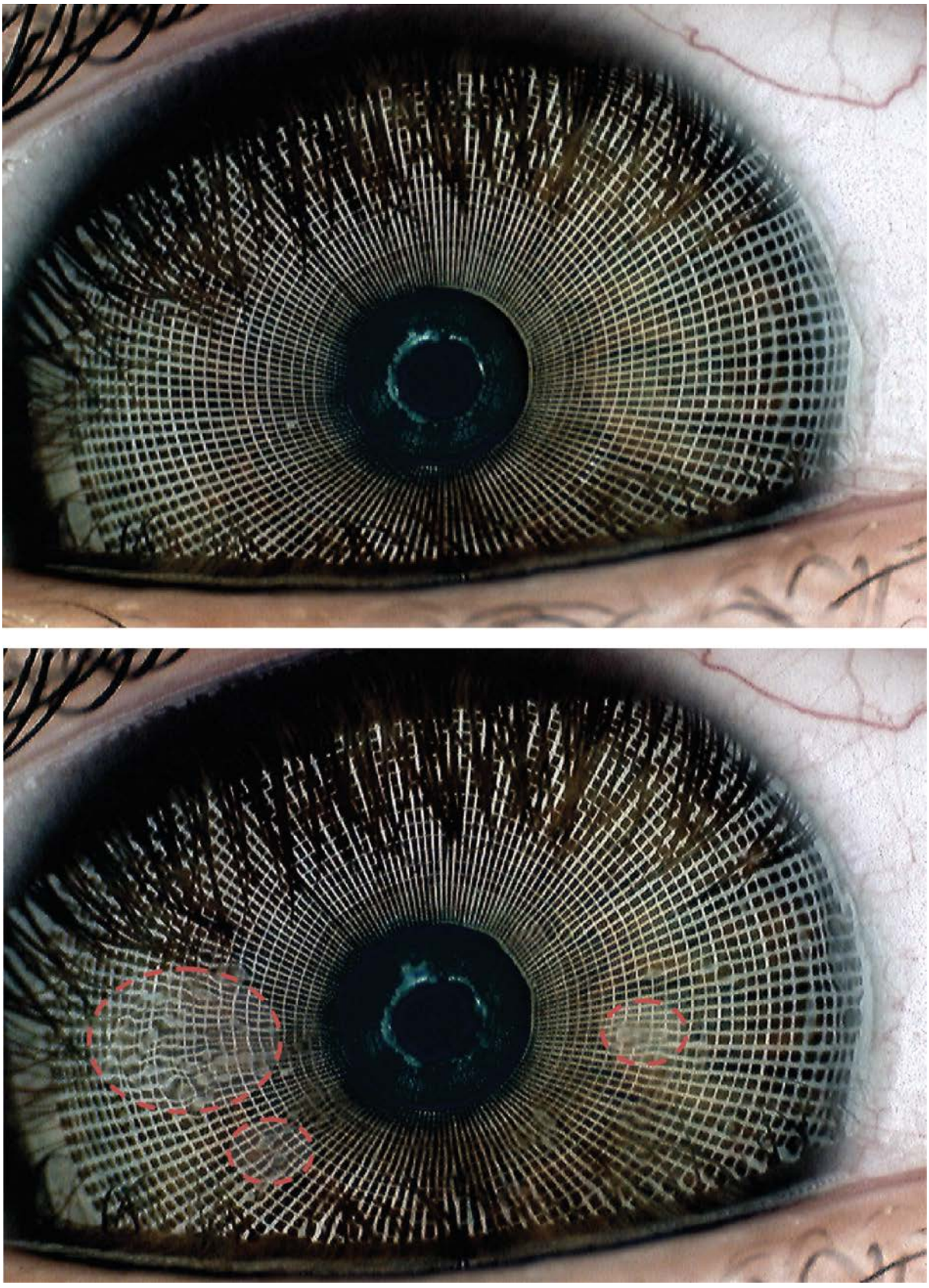

Figure 26: Non Invasive Tear Film Break Up Time Measurement; Top: continuous pre-ocular tear film; Bottom: Tear film with ruptures

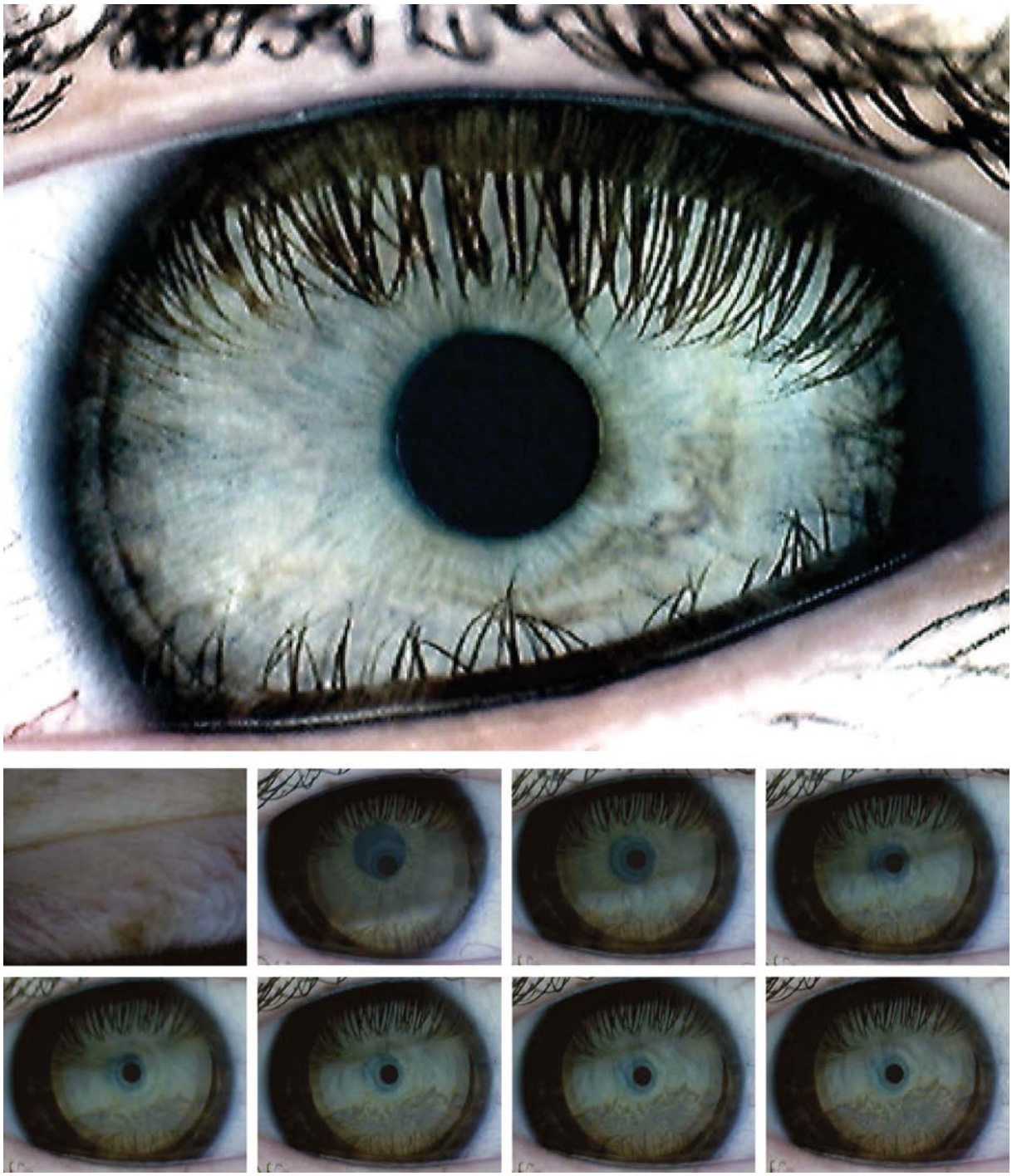

Figure 27: top: Dark areas indicate the variations in lipid layer thickness; bottom: image series of lipid layer movement following a blink

5.1 The illumination unit

The purpose of the illumination unit is the homogeneous, evenly distributed illumination of the corneal surface. The circular illumination is ensured through 36 white LEDs which are equally distributed around and along the device (Figure 28 C,D). The physical principle is the

so called white light interferometry.

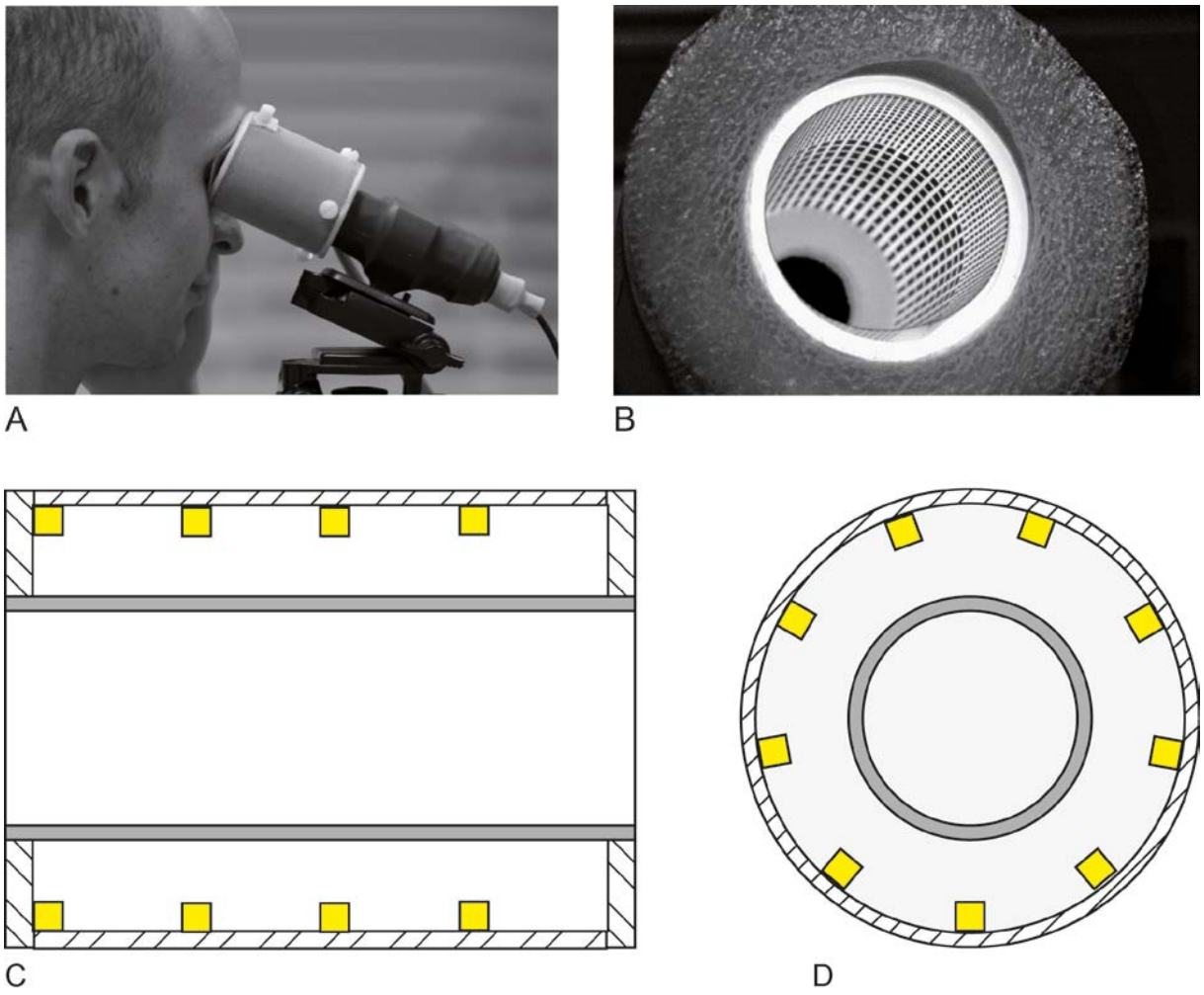

Figure 28: (A) correct placement of the device during the investigation; (B) a close up image of the opening of the device; (C) lateral view of the inside of the device; (D) frontal view of the inside of the device; yellow color indicates the LEDs; dark gray color indicates the diffuse acryl glass tube

Interference is the superimpostion of two or more signals. The resulting signal thereby gets magnified (constructive interference, Figure 29) or ,at least partly, absorbed (destructive interference).

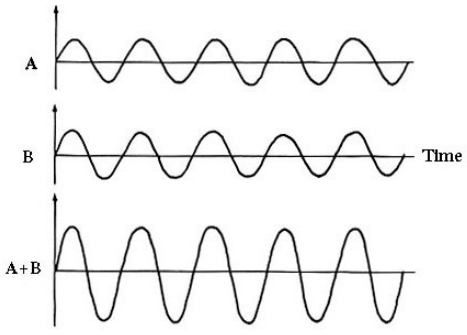

Figure 29: Constructive Interference

The principle of interference is mainly used to determine the thickness of different layers of a substance by the usage of a typically coherent radiation light source. A coherent radiation means the spreading of electromagnetic waves in the same direction, with the same phase and with the same frequency. Such a light source is for example represented by a laser with a narrow bandwidth. On the other hand a light bulb typically emits diffuse light in different directions, at different wavelengths and with different frequency. The first step to investigate the thickness of especially thin layers of fluid is the thin film interferometry. Thereby a coherent light source is directed against the sample. The incoming light beams get partly reflected by the first layer and partly refracted by the passage of the two media with different refractive indices (Figure 30, A). The refracted part of the light beam travels further through medium two and is then reflected at the lower border (Figure 30, B).

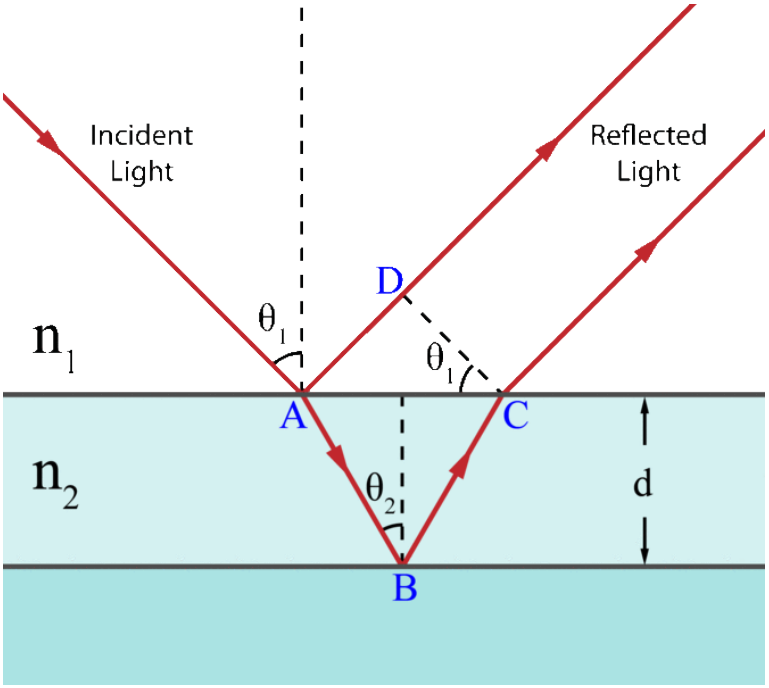

Figure 30: Principle of thin film interferometry

The reflected beam is the refracted again at the border of medium 2 and medium 1, again dependent on the refractive indices of the two media. Because of the difference in the travelled distance the two reflected light beams have a difference phase. This difference in phase then leads to a distinct resulting electromagnetic wave with a defined chromatic appearance, dependent on the superimposition of these two phase-different beams. This phase difference is then proportional to the thickness of the medium.

Two factors contribute to the visibility of the interference fringes: the thickness of the layer and the length of coherence of the illumination source. The length of coherence means the maximum phase and frequency difference of two waves to result in a superimposed wave with a time and spatial stable interference pattern.

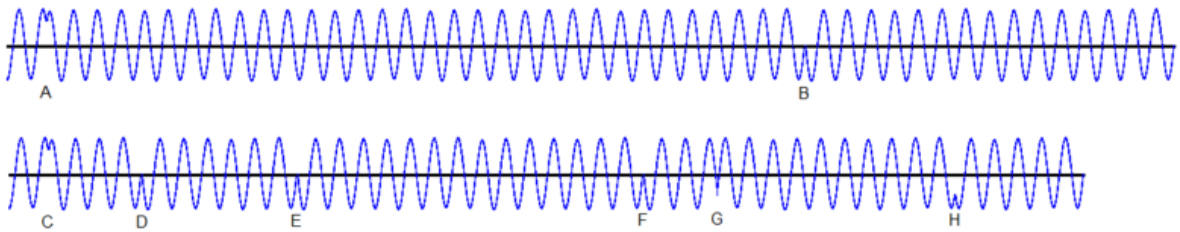

Figure 31: Top: Superimposition of two waves with long length of coherence; bottom: superimposition of two waves with short length of coherence; A-H indicate destructive interference affecting phase and/or amplitude of the resulting signal

When the phase difference of the two signals exceeds their length of coherence, a stable interference pattern does not occur and the resulting signal starts loosing visibility. The spectral distribution of the light source of the LipidViewer is can be divided into a part with a relatively short length of coherence at a high intensity (between approximately 420 and 480 nm) and a large part with a long length of coherence and relatively low emission intensity (approximately from 480 to 700 nm).

A special case is the usage of a broad band white light source like it is used in the LipidViewer.

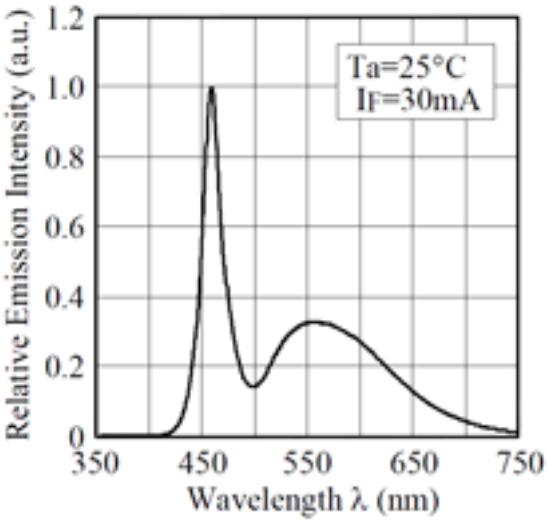

Figure 32: Spectrum of the white LEDs

The distinct peak between approximately 420 and 480 nm results in a length of coherence of 3.051 microns, which results in a maximum measureable lipid layer thickness of 1.141 µm. The visibility diminishes at thicknesses above this value.

The remaining spectral distribution at approximately 480 to 700 nm leads to a length of coherence of 0.763 microns and a visible pattern at a maximum thickness of the lipid layer of 0.285 µm (J.-P. C. Guillon 1987).

Besides the two factors of the length of the coherence of the illumination source and the thickness to be measured, a main factor for the visibility of the interference fringes is the reflectance of the fluid, dependent on the refractive indices of the different layers.

Medium	**Refractive Index**
Air	1
Lipid	1.482
Aqueous solution	1.337

Table 5: Refractive indices within the tear film (J.-P. C. Guillon 1987)

A maximum reflection factor arises at a thickness and a phase shift of cos Φ = 1. A minimum reflection factor thereby occurs at a cos Φ = -1 of the two waves. The values for the maximal and minimal reflection factors are 5.91 % and 2.08 %, respectively. (J.-P. C. Guillon 1987).

5.2 Control Unit

The purpose of the control unit is the continuous control of the LED current and thus the illumination of the eye. From the technical point of view it is desirable to illuminate the eye as high as possible. The reasons are the low reflective factors and thus the low reflected intensity on the CCD chip and which have a negative effect on the exposure time. If this factor of the image acquisition gets too high, minor movements of the eye lead to a motion blurring of the image (Figure 33).

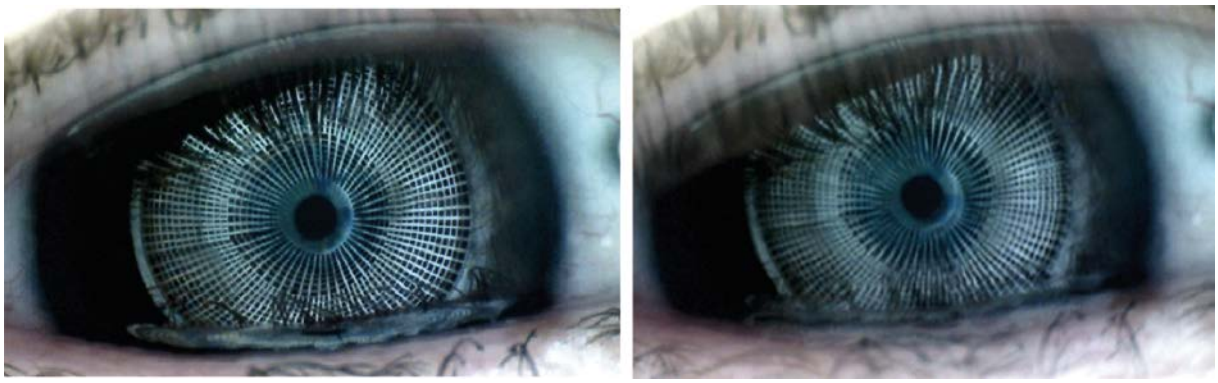

Figure 33: Comparison of focussed image and distinct grid pattern (left) and blurred image because of long exposure time and motion (right)

The input of the control unit is connected with the 5V USB connector of the laptop; the output of the control unit is wired with the four LED strings (9 LEDs each, Chapter 5.1, Figure 34 top). The electrical circuit is shown in Figure 34 (bottom).

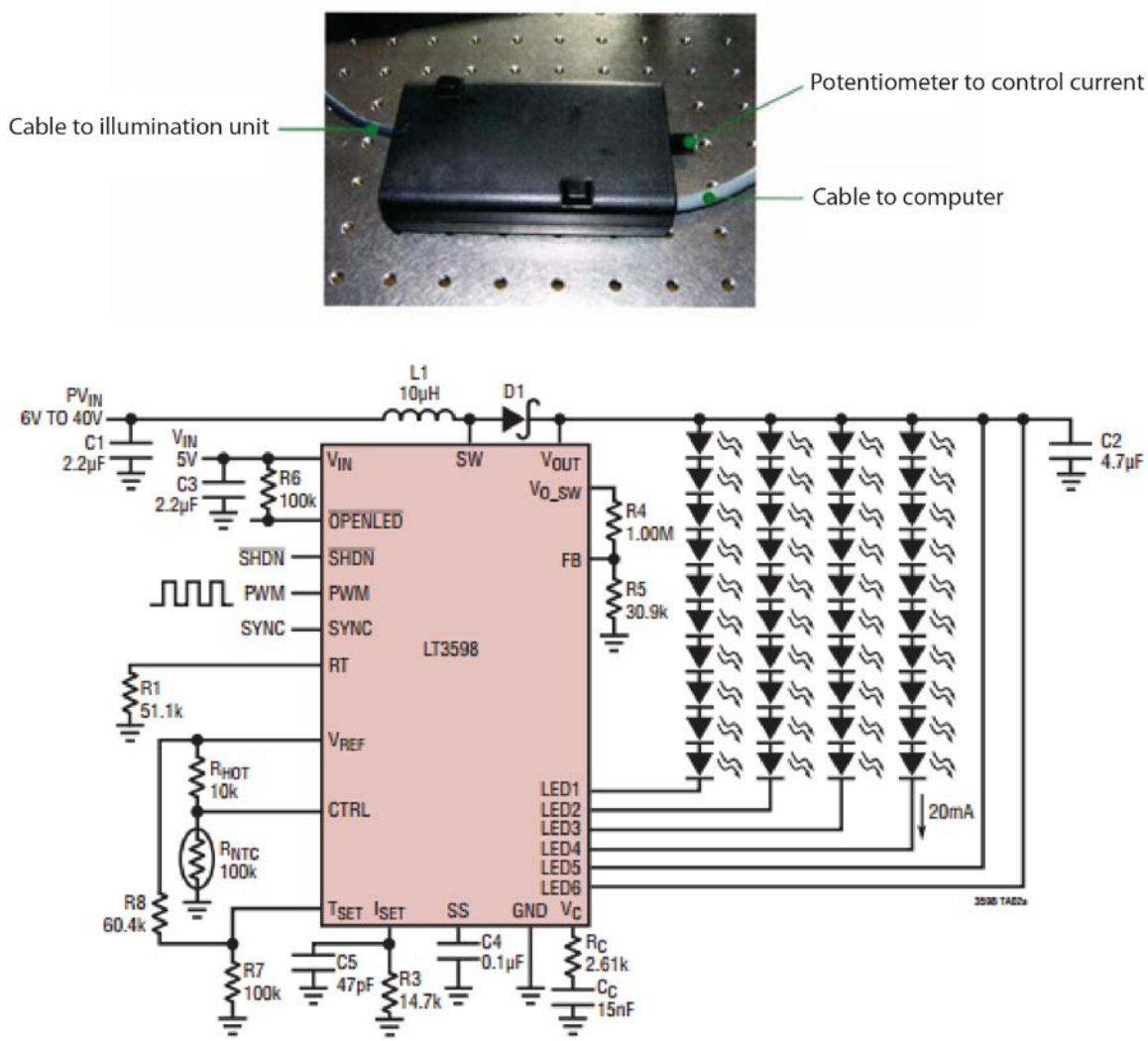

Figure 34: (top) Control unit; (bottom) Electrical curcuit of the LED driver

5.3 Camera

The purpose of the camera (PCE-MM200 Microscope, PCE Group, Meschede, Germany) is to acquire images of the dynamics of the ocular surface. It is mounted at the back of the illumination unit; the user manually has to adjust the focus until the desired feature gets in focus (either the grid of the tear film lipid layer). The specification of the USB microscope is summarized in Table 6.

Figure 35: USB Microscope PCE-200

Max. Spatial Resolution	1600 x 1200
Magnification	10 … 200
Color Depth	24 bit RGB
Max. Temporal Resolution	30 frames per second
Size	110 x 33 mm
Weight	90 g

Table 6: Specification of the USB Microscope PCE-200

5.4 Insulation Transformer

The insulation transformer (ERT 230/230/4/G, Thalheimer Transformatorenwerke GmbH, Thalheim, Germany) fulfills the function of the galvanic insulation of the LipidViewer from the main power supply. This is especially important in order to avoid a possible coupling of critical peaks of the main power supply with the supply of the medical device.

Figure 36: Insulation Transformer; (left) front view; (right) back view

Max. Temperature	40 ° Celsius
Input	230V 50/60 Hz
Output	230 V, 1.9 A
Degree of Protection	IP 20
Protection Category	I
Weight	6.6 kg
In compliance with EN 60601-1	

Table 7: Specification of the Insulation transformer

The laptop (Lenovo S12, Morrisville, NC, USA) displays and saves the acquired images. The leakage current was measured to be below the electrical safety limit for medical devices in accordance with the harmonized family of norms IEC 60601.

Figure 37: Laptop Lenovo S12

Intel Atom N270 CPU
12’’ display, Max. Resolution : 1280 x 800 pixels
1 GB DDR2-533 RAM
250 GB HDD
NVIDIA ION Chipsatz

Table 8: Specification of the Laptop

6 Regulatory framework

This chapter provides the reader with information on the most important laws and technical norms that have to be taken into consideration when developing a medical device. The chapter first explains the regulatory framework at the European level and its translation into national law. It then covers the formulation of the intended use, the classification process of medical devices and the risk management process according to *ISO 14971:2007 Medical Devices – Application of risk management to medical devices [Ref]*. The theoretical background is extended by the practical realization of these regulatory requirements during the technical development of the medical device for the LipidViewer.

6.1 European directives

Three directives regulate the life-cycle of medical devices on the European level (Figure 38).

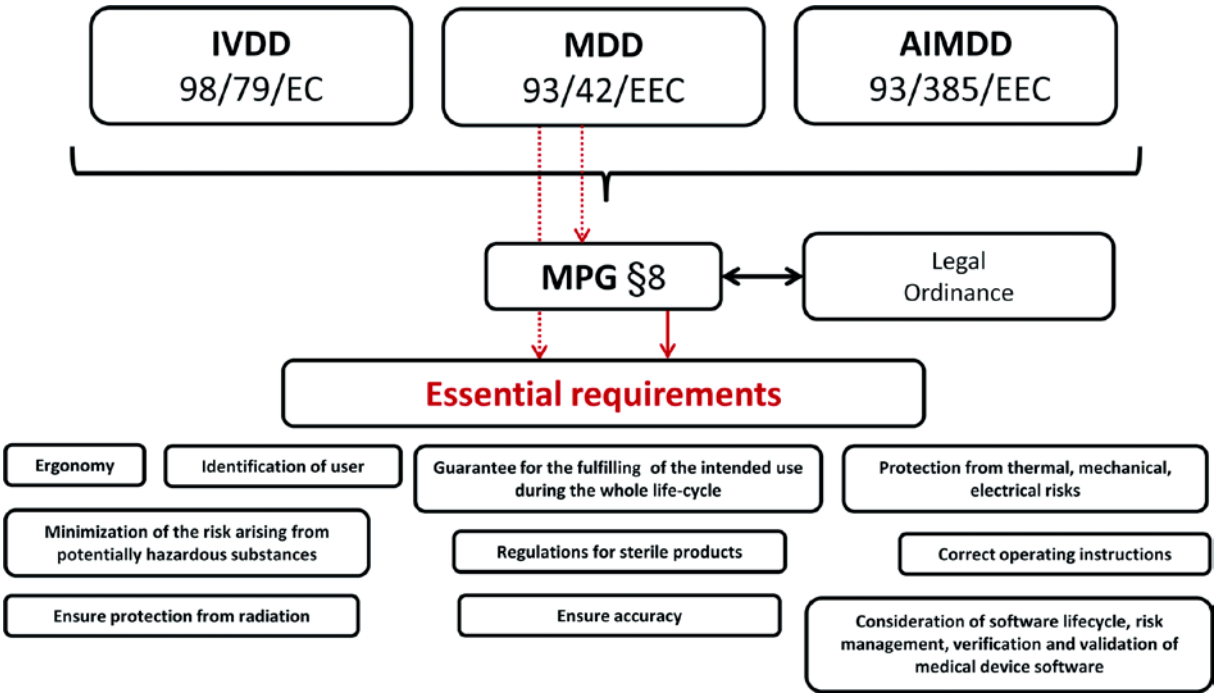

Figure 38: Connection of European directives relevant for medical devices and the national law

MDD 93/42/EEC: The main intention of the *Council Directive 93/42/EEC concerning medical devices* (MDD *93/42/EEC)* is the regulation of the free movement of goods of the medical device market within Europe. This directive forms the basis for the development of medical devices and covers important topics like “Essential Requirements”, “Classification”, “Clinical Evaluation” and the different conformity assessment procedures. Important updates

covering the software-lifecycle and the clinical trial have been made through an amendment in 2007.

IVDD 98/79/EC: The directive *98/79/EC of the European Parliament and of the Council on in vitro diagnostic medical devices* covers the life-cycle of laboratory medical devices intended to perform analytical in vitro diagnosis in order to observe organic processes outside the human organism. The main difference compared to the MDD 93/42/EWG is the focus on the measurement and analytical procedure.

AIMDD 93/385/EEC: The *Council Directive 93/385/EEC on active implantable electrical devices* covers the life-cycle of medical devices which *"are intended to be totally or partially introduced, surgically or medically, into the human body or by medical intervention into a natural orifice, and which is intended to remain after the procedure".* The definition of active medical devices is *"any medical device relying for its functioning on a source of electrical energy or any source of power other than that directly generated by the human body or gravity"*.

In Austria these three directives are embedded into national laws by the medical device law (Medizinproduktegesetz, MPG). However, these European directives define only a minimum standard for the applied national law. More concrete, the communication between national authorities, the manufacturer and the user is regulated in a way that technical safety representatives and medical device advisors have to be installed by the manufacturer. The technical safety representative can be seen as the first contact point for users of the medical device if any potential harmful situation arises within the usage of the medical device. The technical safety representative then has to ensure the correct communication between the user and the manufacturer and report the hazardous situation to the authorities.

The medical device advisor is responsible for the information of the user concerning any questions about the intended usage of the medical device. Both positions have to be registered at the *Austrian Medical Device Register* (Ref).

In addition to the MPG several national legal ordinances have been published (e.g. the medical operator ordinance regulates the income test, the maintenance and the periodically safety related technical check).

6.2 Essential Requirements

An especially for the technical development crucial chapter in the MDD regulates the essential requirements for medical devices. They are defined in Annex 1 of the MDD and in §8 of the MPG and deal amongst others with aspects of ergonomics, hygiene or the user manual.

In general, the essential requirements are thought to ensure that the *devices must be designed and manufactured in such a way that, when used under the conditions and for the purposes intended, they will not compromise the clinical condition or the safety of patients, or the safety and health of users or, where applicable, other persons, provided that any risks which may be associated with their use constitute acceptable risks when weighed against the benefits to the patient and are compatible with a high level of protection of health and safety* (Ref).

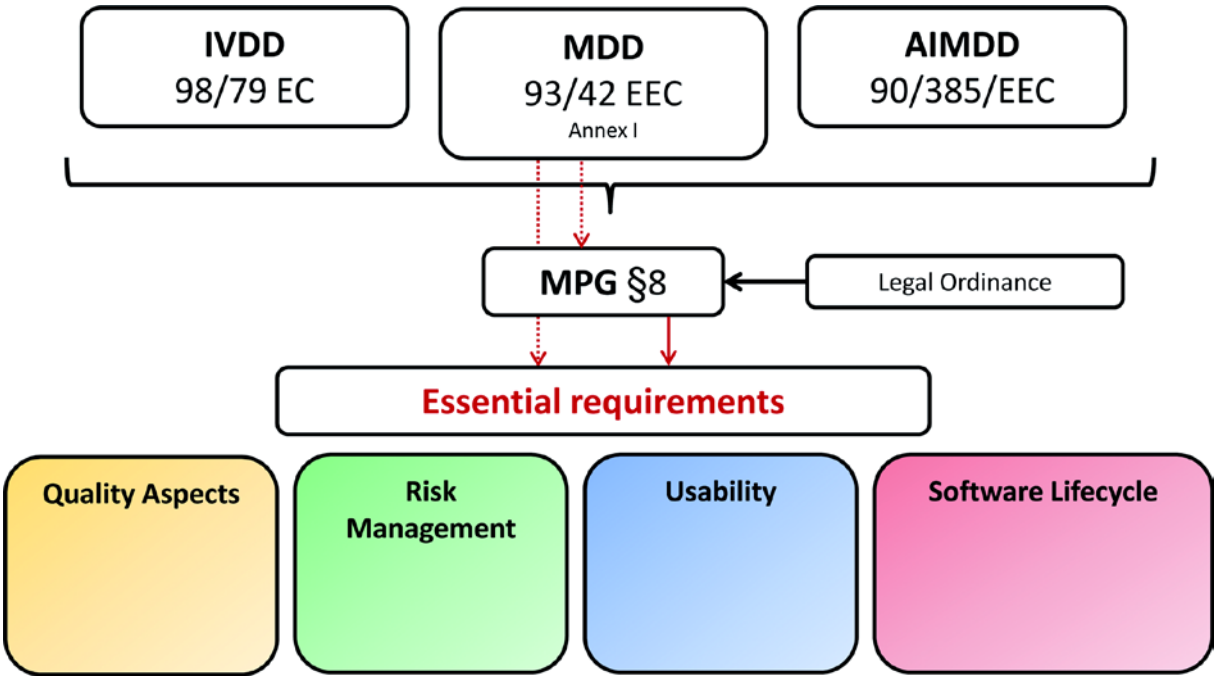

Figure 39: Categorization of the essential requirements

Due to the fact that the essential requirements are phrased in general terms, the concept of harmonized norms was established at the European level. The principle is that detailed harmonized norms regulate the fulfilling of the respective essential requirement. Therefore the manufacturer of the medical device has to prove the conformity with each of these harmonized norms in order to fulfill the essential requirement for medical devices.

A categorization of the essential requirements can be seen in Figure 39. The translation of these categories in harmonized norms is shown in Figure 40.

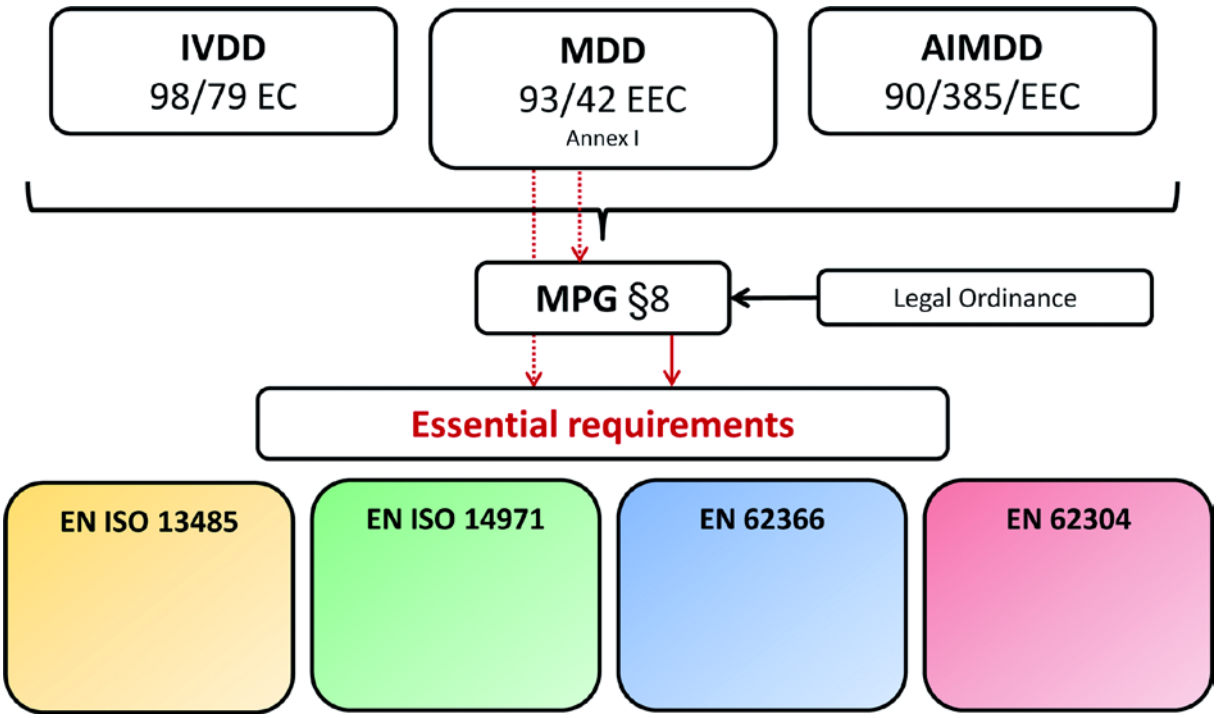

Figure 40: Harmonized norms to fulfill the essential requirement for medical devices

It is important to note, that the harmonized norms in Figure 39 and Figure 40 are not the only ones the conformity have to be ensured with. Besides these, product group specific norms have to be taken into consideration. An exceptional position thereby has the IEC 60601 family of norms which regulates among other points the electrical safety of active medical devices, e.g. concerning the electromagnetic radiation or leakage currents (Ref).

6.3 Conformity assessment procedures

For medical devices according to 93/42/EEC the conformity with the norms can be proven by several conformity assessment procedures. An essential basis for the correct type of conformity assessment procedure is the correct classification of the medical device. The classification criteria are defined on European level in the 93/42/EEC, Annex IX. Medical devices are grouped according to their potential risk in the following classes: I, Im, Is, IIa, IIb and III. This classification is performed by the application of 18 rules (Rule 1-4: not invasive products; Rule 5-8: invasive products; Rule 9-12: additional rules for active medical devices; Rule 13-18: special rules). Criteria for the classification are among others the duration of the usage, the invasiveness or the required sterility.

The possible conformity assessment procedures for the different classes of medical devices are shown in (Figure 41).

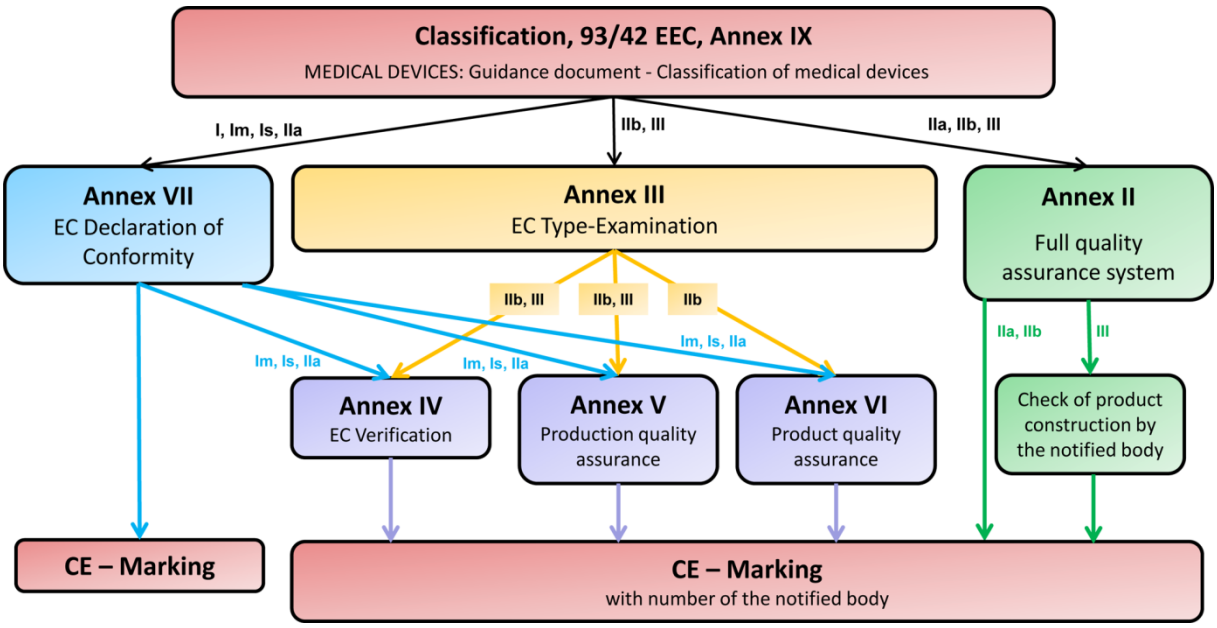

Figure 41: Overview of the different conformity assessment procedures

The different conformity assessment procedures are underlying a simple principle: the manufacturer has to ensure that the construction of the product is conforming to the harmonized norms. In addition to this the manufacturer has to prove that its organization is able to produce the product thus that the product quality can be ensured for a larger quantity. To ensure that the production process is implemented correctly, the manufacturer has to choose between three options:

[1] Check a number of the medical device after the production process,

[2] check if the production process is in conformity with the norms and

[3] check if the process checking the number of medical devices is legitimate (e.g. correct sample size, randomization).

It is only valid for medical devices of class I to prove the conformity with the essential requirements by a declaration of conformity issued by the manufacturer. As an alternative to the previous described two-staged process of conformity assessment procedure, it is also possible to implement a full quality assurance system. The basement of such a quality assurance system is the harmonized norm ISO 13485:2003 Medical Devices – Quality management systems – Requirements for regulatory processes [Ref].

6.4 Intended use of the LipidViewer

As mentioned before, the definition of the intended use is a prerequisite for the correct classification of the medical device. The intended use for the LipidViewer is defined as following:

The LipidViewer is an active, diagnostic device intended to create videos of the tear film dynamics on the ocular surface. The generated videos are further on subjectively graded by the user in order to manually acquire parameters on the performance of the tear film lipid layer.

The medical device is intended to be used within clinical environment. The investigation is performed non-invasively without touching the ocular surface. The users are ophthalmologists.

The investigation lasts approximately four minutes; the skin around the eye thereby gets in contact with non-metal parts of the LipidViewer. During the investigation, the eye of the patient is illuminated with light in the visible light spectrum.

According to 93/42/EEC, Annex IX, Rules 10 and 12 the LipidViewer can be classified as a medical device of class I.

6.5 Conformity assessment procedure

Due to the intended use as aforementioned, the process as described in *93/42/EEC, Annex VII – EC Declaration of Conformity* will be used to document the compliance with the essential requirements for medical devices.

6.6 Risk management process

The risk management process for medical devices is defined in the norm *ISO 14971:2007- Application of risk management for medical devices (Ref)*. The first chapter explains the motivation and scope of the norm. The second chapter covers terms and their definition. The third chapter defines the general requirements for the risk management, including management responsibilities, qualification of the personnel responsible for the risk management, content of the risk management plan and of the risk management file. The risk management process itself is described in the following chapters, starting with the risk analysis. Two concrete examples are given for each task.

6.6.1 Identify possible hazards

The first step is to identify possible hazards arising from both normal and reasonably foreseeable misuse. Annex C in the *ISO 14971:2007* therefore contains questions which assist this task.

Example 1:

Question C.2.5: Is energy delivered to or extracted from the patient? Factors, that should be considered include:

[a] the type of energy transferred;

[b] its control, quality, quantity, intensity and duration;

[c] whether energy levels are higher than those currently used for similar devices.

To exclude phototoxic effects, the illumination of the LipidViewer has to be taken into consideration for further risk management activities.

Example 2:

Question C.2.16 :

Does the medical device influence the environment? Factors, that should be considered include:

[a] the effects of power and cooling supplies;

[b] emission of toxic materials;

[c] the generation of electromagnetic disturbances.

In our case, the LipidViewer emits electromagnetic energy. This identified risk is taken into consideration for further risk management activities.

6.6.2 Estimation of the risk(s) for each hazardous situation

The main task within this activity is the identification of reasonable foreseeable sequences or combinations of events which could potentially lead to harm to the user, the patient and/or the environment.

Given this sequence for events, the risk can be quantified by considering the probability and severity of the damage. A possible way to categorize those two aspects can be found in Annex D of the ISO 14971:2007 and is shown in Table 9 and Table 10.

Terms	**Description**
Catastrophic	Results in patient death
Critical	Results in permanent impairment (blindness)
Serious	Results in injury or impairment requiring professional medical intervention
Negligible	Inconvenience or temporal discomfort

Table 9: Qualitative criteria of the severity of the potential harm of the risk

Terms	**Description**
High	Likely to happen, often, frequent
Medium	Can happen, but not frequently
Low	Unlikely to happen, rare
Negligible	Probability towards zero

Table 10: Qualitative criteria for the probability of the risk

The evaluation of the risk according to both probability and severity can be seen in Table 11.

Example 1: Risk due to the illumination

Example 2: Risk due to the electromagnetic disturbances

High				
Medium			**Example 1**	
Low				**Example 2**
Negligible				
	Negligible	Serious	Critical	Catastrophic

Table 11: Estimation of the risk of example 1 and 2

6.6.3 Risk evaluation

Based on the criteria as defined in the risk management plan, the manufacturer then has to decide whether risk control measures have to be taken into consideration in order to control

the risk and bring it to an acceptable level. In our case, both example 1 and 2 need a risk control measure.

6.6.4 Risk control option analysis

For each of the identified risks control measure an analysis of the risk control options has to be performed, according to the following sequence of priorities:

[a] inherent safety by design;

[b] protective measures in the medical device itself or in the manufacturing process;

[c] information for safety.

Risk control options for Example 1 – Illumination of the eye

Inherent safety by design

The led module is designed such that the current driving the LEDs cannot exceed 15mA. This value can be considered to be low enough not to harm aphakic eyes unless the illumination time is below 38.5 minutes (*according to EN ISO 15004-2:2007 Fundamental requirements and test methods – Part 2: Light hazard protection*). Therefore a certified laboratory has to measure and ensure a proper

Information for safety

According to *EN ISO 15004-2:2007,* a short text has to be included in the manual, stating that the limit of the duration of the investigation at full illumination must not exceed 38.5 minutes.

6.6.5 Residual risk evaluation

The next step is to evaluate the residual risk after the risk control option implementation:

High				
Medium				
Low				
Negligible			**Example 1+2**	
	Negligible	Serious	Critical	Catastrophic

Table 12: Estimation of the residual risks of example 1 and 2

6.6.6 Risk/benefit analysis

If the residual risk is not judged acceptable using the criteria established in the risk management plan and further risk control is not practicable, the manufacturer may gather and review data and literature to determine if the medical benefits of the intended use outweigh the residual risk.

This point is not applicable for our examples.

6.6.7 Risks arising from risk control measures

7 Clinical Trial

This chapter provides a detailed explanation of the study design, the documents necessary for the positive vote of the ethics commission and the submission to the Austrian medical device surveillance agency (AGES).

7.1 Study design

The study was conducted as a prospective, clinical trial to compare the parameters acquired with the LipidViewer with common diagnostic procedures. The correlations between the novel and conventional parameters were thereby checked for significance.

7.2 Subjects

Participants were chosen from the dry eye unit and from the staff of the Department of Ophthalmology, Medical University Graz. The experiments were approved by a local ethics committee and were in accordance with the 1964 Declaration of Helsinki. All subjects gave their informed consent to participate prior to their inclusion in the study. A total of 59 subjects (n= 45 female, n= 14 male) were included in the study. The subjects were divided into three subgroups, based on their subjectively reported severity as determined with the Ocular Surface Disease Index (OSDI©) (Schiffman, et al. 2000): Subgroup 1 (mild, OSDI©: 0-15, gender: 15/4 f/m, age: 55.2 ± 16.4 a), Subgroup 2 (moderate, OSDI©: 16-30, 11/6 f/m, 52.9 ± 13.6 a), Subgroup 3 (severe, OSDI©: 31-100, 19/4 f/m; 58.65 ± 13 a). The distribution of the study population is shown in Figure 42.

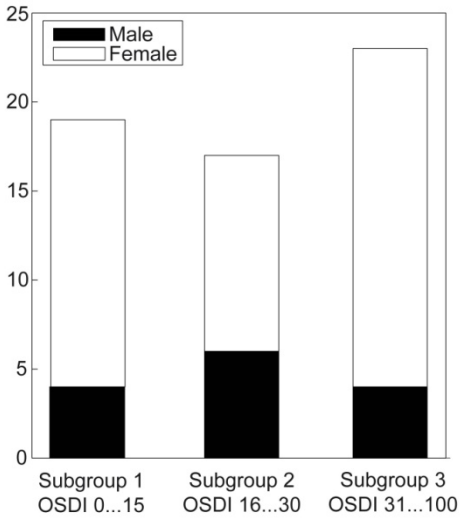

Figure 42: Distribution of subjects

7.2.1 Exclusion / Inclusion Criteria

Subjects were excluded from the study if they showed symptoms of an active inflammation of the eye, any types of lid deformation or if they had had ocular surgery within six months before the study took place. Subjects were instructed not to use artificial tears or other local medications relevant to the tear film for at least 2 hours before the investigation. Contact lens wearers (n= 5) removed their contact lenses at least 8 hours prior to their examination.

7.3 Statistical Analysis

The correlation of the corrected and uncorrected lipid layer stabilization time with the other clinical parameters was determined through the calculation of Spearman's rank correlation coefficient. A linear regression analysis was conducted to quantify the relationship of the corrected lipid layer stabilization time and the other parameters.

Both the corrected and uncorrected lipid layer stabilization time as well as the OSDI© score were checked for normality (Kolmogorov–Smirnov, $p > 0.05$; Q-Q- Plot). Since normality was not present, the non-parametric Wilcoxon signed rank test was used to check for significant differences between groups of different subjective severity. Significance level was set to $p<0.05$ for significant correlations, and $p<0.01$ for highly significant correlations. Statistical Analysis was performed with the Statistics Toolbox of Matlab (MathWorks, Matlab, Natick, MA, United States).

7.4 Methods

To characterize the subjects, tests were performed in the following sequence:

Tear osmolarity was measured with the Tearlab® Osmolarity System (Ocusense, San Diego, CA, United States). A small sample of tear fluid was taken from the lower tear meniscus of each patient using a pen. The bottom of the tip thereby came into contact with the thin line of moisture between the lower eyelid and the eye. Fluid was collected at the bottom tip of the test card and the result was displayed after a few seconds. The measurement range is linear from 275-400 mOsms/L.

TFBUT was measured by touching the inferior temporal bulbar conjunctiva with a fluorescein sodium strip, wetted with a preservative-free isotonic sodium chloride solution. Patients were instructed to blink. The pre-corneal tear film was then examined under blue-light illumination using a biomicroscope with a 10-fold magnification. The mean value of a total of three measurements was recorded.

Sterile strips impregnated with **lissamine green** (HUB Pharmaceuticals, LCC Rancho Cucamonga, CA, United States) were used to classify the exposed interpalpebral portions of the nasal and temporal conjunctiva and the cornea. The extent of staining was graded according to the van Bijsterveld score 0-3 (0, negative; 1, scattered minute; 2, moderate spotty; and 3, blotchy) for each zone with a maximum score of 9 27.

A 5-minute conventional **Schirmer test** without anesthesia was performed on closed eyes by placing a commercially available 5 x 35 mm paper strip (Haag-Streit, Harlow Essex, UK) over the lid margin at the junction of the middle and lateral third into the tear film.

8 Abbreviations

DED … Dry Eye Disease

KCS … Keratoconjunctivitis sicca

IVDD … In-vitro Diagnostic Directive

MDD … Medical Device Directive

AIMDD … Active Implantable Medical Device Directive

MPG … Medizinproduktegesetz

9 Outlook

This chapter explains future research directions based on DED and the corrected lipid layer stabilization time. The outlook can be divided into scientific and technical aspects. Concrete examples for future studies of potential interest are therefore described as well as technical solutions to enhance both the functionality and usability of this medical device.

9.1 Study Design I

Title: Influence of the viewing size on the spontaneous eyeblink activity during visual display terminal usage.

9.1.1 Authors

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9.1.2 Executive Summary

Subjects working excessively in front of a computer screen often report about burning, dryness and other signs of Dry Eye Disease (DED).Thus, tired and strained eyes have been reported to be the most common cause of disruption of office work (C. G. Begley, B. Caffery, et al. 2002). Besides these subjectively reported symptoms of dry eye disease during visual display usage, the objectively assessed integrity of the tear film has also been shown to decrease with cognitive demanding tasks in front of a computer screen (Cardona, et al. 2011). A number of environmental factors have yet been identified to be potential risk factors for an unstable tear film. These include a high room temperature (Mendell, et al. 2002) and/or reduced air humidity (Tsubota, Hata, et al. 1997). Another proposed factor is

the change of spontaneous blink activity (SBA) during visual display terminal usage. An efficient (= complete, quick) blink is responsible for a proper distribution of the pre-ocular tear film on the exposed corneal surface epithelia (Benedetto, Clinch und Laibson 1984) (Korb, et al. 1994) (Linton, Curnow und Riley 1961) (McMonnies 2007). Since the blink frequency as well as the blink amplitude significantly decline during visual display terminal usage, symptoms of dry eye are among the most prominent symptoms reported as a result of excessive computer work (Uchino, et al. 2008). It has been proposed, that a magnification of the displayed letters and symbols can lower the cognitive load, increase the SBA and thereby lower the interblink interval.

Thus, the underlying hypothesis of the current study is that the influence of the displayed size of letters or symbols correlates with the SBA. Within this study the SBA of 50 young subjects with mild to moderate symptoms of dry eye during two trials are analyzed. These two sessions have the same cognitive demand but different viewing sizes.

9.1.3 Hypothesis

Does the overall blink frequency significantly increase by a magnification of 200% of the digital workplace?

Does the blink frequency of complete blinks (=covering more than 2/3 of the corneal surface) significantly increase by a magnification of 200% of the digital workplace?

Does the blink frequency of incomplete blinks (=covering less than 2/3 of the corneal surface) significantly increase by a magnification of 200% of the digital workplace?

Does the maximum down phase velocity change significantly by a magnification of 200% of the digital workplace?

Does the down phase duration change significantly by a magnification of 200% of the digital workplace?

Does the steady phase duration change significantly by a magnification of 200% of the digital workplace?

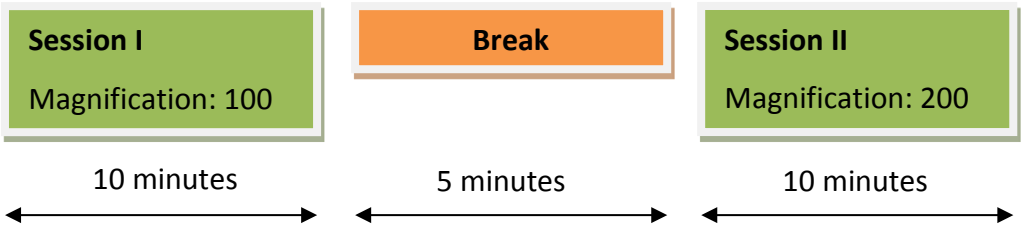
Does the maximum up phase velocity change significantly by a magnification of 200% of the digital workplace?

Does the upward phase duration change significantly by a magnification of 200% of the digital workplace?

9.1.4 Study Design

The study is designed as a prospective, interventional study.

9.1.5 Flow Chart



9.1.6 Setting

The measurements will be conducted in the office of Michael Ring. During each of the sessions, the subjects are wearing the EyeSeeCam System. It has to be ensured that the room temperature and air humidity has to be kept fairly constant (e.g. no open windows during the measurement). The illumination of the room has to be kept at a constant level (e.g. shut the sunblind, turn on office light). The viewing distance will be 50 cm; the level of the eye in upright posture is the same level as the upper edge of the monitor.

9.1.7 Sample Population

The subjects (n=50) will be recruited from the student population. Since blink patterns change with age the age of the subject is restricted to be below 35. No care has to be taken about gender differences of SBA.

9.1.8 Inclusion Criteria

- Age between 20 and 35
- OSDI < 20
- No contact lens wearing 8 hours prior the sessions and during the sessions
- No acute inflammation of the ocular surface
- No lid abnormality
- No wearing of make up at the day of evaluation
- No correction of visual acuity

9.1.9 Intervention

In both sessions, the subjects have to insert spaces after each word of a text. The break of 5 minutes between two sessions can be seen as a resting period.

9.1.10 Parameters

Overall blink frequency [1 / min]

Overall blink frequency, complete [1/min]

Overall blink frequency, incomplete [1/min]

Maximum velocity of downward movement [mm/sec]

Duration of downward movement [msec]

Duration of steady phase [msec]

Maximum velocity of upward movement [mm/sec]

Duration of upward movement [msec]

9.1.11 Statistical Evaluation

Data will be checked for normal distribution with the Kolmogorov-Smirnov test. If the data is normal distributed, the significancy will be checked by the paired t-test. If not, the Wilcoxon-Signed rank test will be used for these purposes. Significance level is set to $p<0.05$ for significant correlations, and $p<0.01$ for highly significant correlations.

9.2 Study design II

Influence of simple blink exercises on the plasticity of lid closure behavior and its relation to tear film integrity in dry eye patients.

9.2.1 Authors:

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Tel.: +43 (0)5 0804 -52170

DI(FH) Michael Ring

FH OÖ Studienbetriebs GmbH

Garnisonstraße 21

4020 Linz/Austria

Tel.: +43 (0)5 0804 -55010

9.2.2 Executive Summary

An efficient (= complete, quick) blink is responsible for a proper distribution of the pre ocular tear film on the exposed corneal surface epithelia (Benedetto, Clinch und Laibson 1984) (Korb, et al. 1994) (Linton, Curnow und Riley 1961) (McMonnies 2007).

Since the blink frequency as well as the blink amplitude significantly decline during visual display terminal usage, symptoms of dry eye are among the most prominent symptoms reported as a result of excessive computer work (Uchino, et al. 2008). Besides the subjectively reported symptoms of dry eye disease, the integrity of the tear film was also shown to decrease with cognitive demanding tasks in front of a computer screen (Cardona, et al. 2011).

Since the blinking behavior has been reported to be conditioned through simple blink exercises (Jenkins, Rehkopf und Brown 1978) (Collins, et al. 1987) it is intuitive to think of simple blink exercises as a possible therapy for dry eye patients. The suggested intensity of these exercises are 30 seconds each 30 minutes for one week (McMonnies 2007), but an objectively assessed improvement as a result of the training procedure has not been published yet. The main purpose of this study is therefore the assessment of the stability of the pre ocular tear film within subjectively reported dry eye patients before and after performing blink exercises.

Additionally the dynamics of the blink action are acquired before and after the training period. Therefore, the eyelid closure behavior is recorded with a high speed camera and analyzed offline.

9.2.3 Hypothesis

Does the invasive tear film break up time change significantly (200%) through the described training paradigm?

Does the lipid layer stabilization time change significantly (-60%) through the described training paradigm?

Do the subjectively reported symptoms change significantly (OSDI score -50%) through the described training paradigm?

Does the maximum down phase velocity change significantly through the described training paradigm?

Does the down phase duration change significantly through the described training paradigm?

Does the steady phase duration change significantly through the described training paradigm?

Does the maximum upward phase velocity change significantly through the described training paradigm?

Does the upward phase duration change significantly through the described training paradigm?

9.2.4 Study Design

The study is designed as a prospective, interventional, randomized, controlled study.

Annex I – Technical Certificates

Electromagnetic Radiation

emv consulting
Elektromagnetische Verträglichkeit -•- Elektrische Sicherheit
Beratung -•- Planung -•- Projektbegleitung
Dipl. Ing. Wilfried Ottinger A-4872 Neukirchen, Hauptstraße 2
+43 (0)7682 - 7609 office@emvconsulting.at

EMV
Teilprüfbericht

Elektromagnetische Verträglichkeit

Report # **EMVC 2011-03-13**

Projekt: **Tränenflüssigkeitsanalysegerät**

Hersteller: **FH-Linz**

1 Prüfobjekt

1.1 Prüflingsidentifikation

Auftraggeber: Fachhochschule Oberösterreich Studienbetriebs GmbH
Franz Fritsch Straße 11 / Top 3
4600 Wels
Tel.: +43 (0)7242 44808-40
Fax: +43 (0)7242 44808-77
Email: research@fh-ooe.at

Kontaktpersonen: Hr. Michael Ring
Tel: 0732 2008 5010
Email: michael.ring@fh-linz.at
Hr. Friedrich Mayr
Mobil: 0676 5265041
Email: friedrich.mayr@fh-linz.at

Prüfobjekt:	Tränenflüssigkeitsanalysegerät
Type:	noch keine Modellbezeichnung
Hersteller:	FH OÖ Forschungs- & Entwicklungs- GmbH
Seriennummer:	Prototyp
Laptop:	HSTNN-I86C-5
Hersteller:	HP
Laptop Netzteil:	(HP Part Nr.) 606421-002
Input:	100-240Vac 50/60Hz 1,7A
Output:	18,5Vdc 3,5A 65W
Seriennummer:	F12931022000165

Betriebsbedingungen: Normalbetrieb

Prüfgrundlage: EN60601-1-2:2007

Ort der Messung: EMV-Labor EMV Consulting A-4872 Neukirchen

Datum der Messung: 15.3.2011

Bemerkungen: Die Messungen wurden in Anwesenheit der Hrn. Mayr und Ring durchgeführt.

Ergebnis: Es wurden nicht alle nötigen Prüfungen durchgeführt, weil das derzeit verwendete Netzteil nicht dem zukünftigen Serienstand entspricht. Abgesehen vom Punkt ESD entspricht das Produkt, im durchgeführten Prüfumfang, den Anforderungen der oben angeführten Norm.

Umgebungsbedingungen: T=21,4 °C, F=33,6%

Durchführung der Messung: Dipl. Ing. Ottinger Willy

Dipl. Ing. Wilfried Ottinger A-4872 Neukirchen, Hauptstraße 2
+43 (0)7682 - 7609 office@emvconsulting.at

2. Zusammenfassung der Prüfungen :

Übereinstimmung mit der Störemissionsanforderung EN60601-1-2:2007

2.1 Störspannungsmessung (50 Ohm/ 50 µH LISN) **gemäß EN55011 Klasse A,**
150 kHz -----30 MHz keine Messung durchgeführt ☐✓

2.2 Elektromagnetische Feldstärke **gemäß EN55011 Klasse A,**
Messentfernung 3m , Umrechnung auf 10 m mittels rückführbaren Hallenfaktors
Voraussetzung: Durchführung der Modifikation_1
10m: 30 - 230 MHz 40 dB µV/m, 230-1000 MHz 47 dB µV/m erfüllt ☐✓ nicht erfüllt ☐
3m: 1000-3000 MHz 50 dB µV/m (AVG), 70 dB µV/m (PEAK) keine Messung durchgeführt ☐✓
3m: 3000-6000 MHz 54 dB µV/m (AVG), 74 dB µV/m (PEAK) keine Messung durchgeführt ☐✓

2.3.1 Netzrückwirkung Stromoberwellen **(EN61000-3-2)**
100 Hz bis 2 kHz, Geräteklasse A nicht anwendbar ☐✓

2.3.2 Netzrückwirkung Flicker **(EN61000-3-3)**
100 Hz bis 2 kHz nicht anwendbar ☐✓

Übereinstimmung mit der Störfestigkeitsanforderung EN60601-1-2:2007

2.4 Elektromagnetisches Feld, moduliert **(EN 61000-4-3)**
80 - 2500 MHz 3V/m, 1kHz (AM) Bewertungskriterium A erfüllt ☐✓ nicht erfüllt ☐
80 - 3000 MHz 3V/m, 1kHz (AM), **informativ** BK: A erfüllt ☐✓ nicht erfüllt ☐

2.6 Magnetfeld bei 50 / 60 Hz (3 A/m) **(EN61000-4-8)**
Bewertungskriterium A erfüllt ☐✓ nicht erfüllt ☐
Magnetfeld bei 50 / 60 Hz (30 A/m), **informativ,** Bk: A erfüllt ☐✓ nicht erfüllt ☐

2.7 Elektrostatische Entladung (ESD) **(EN61000-4-2)**
2,4,6 kV Kontakt Bewertungskriterium B erfüllt ☐✓ nicht erfüllt ☐
2,4 kV Luft Bewertungskriterium B erfüllt ☐✓ nicht erfüllt ☐
8 kV Luft Bewertungskriterium B erfüllt ☐ nicht erfüllt ☐✓

2.8 Schnelle Transiente (BURST) **(EN61000-4-4)**
auf Signal- und Steuerleitungen
1,0kV Puls 5/50ns, 5 kHz, kap. Koppelzange, Bewertungskriterium B keine Messung durchgeführt ☐✓
auf Netz Ein / Ausgänge Wechselstrom
2,0kV Puls 5/50ns, 5 kHz, Bewertungskriterium B keine Messung durchgeführt ☐✓

2.9 HF-Einkopplung (150kHz-80MHz) AC Input, **(EN61000-4-6)**
150 kHz-80 MHz 3Vemk, 1kHz 80% AM Bewertungskriterium A keine Messung durchgeführt ☐✓

2.10 Stoßspannungen AC Versorgung (SURGE) **(EN61000-4-5)**
1000V Puls 1,2/50 (8/20) µs Ri 2 Ohm L zu N, Bewertungskriterium B keine Messung durchgeführt ☐✓
2000V Puls 1,2/50 (8/20) µs Ri 12 Ohm L/N-PE, Bewertungskriterium B nicht anwendbar ☐✓

2.11 Spannungseinbrüche/ Spannungsunterbrechung **(EN61000-4-11)**
10ms, 100% (B), 500ms 30% (C), 5sec 100% (C)
Bewertungskriterium (B, C) keine Messung durchgeführt ☐✓

9.3 Illumination

SEIBERSDORF LABORATORIES

STAATLICH AKKREDITIERTE PRÜFSTELLE (NR. 312)
für Laser und Optische Strahlung

PRÜFBERICHT NR. LE-L-0185-1/10

Über:	Prüfung eines Tränenfilmanalysators gemäß EN ISO 15004-2:2007
Auftraggeber:	FH Oberösterreich Forschungs & Entwicklungs GmbH F&E Medical Engineering
Adresse:	Garnisonsstraße 21 4020 Linz
Prüfgegenstand:	Tränenfilmanalysator (Prototyp)
Ergebnis:	Beim geprüften Tränenfilmanalysator handelt es sich um ein ophthalmisches Instrument der Gruppe 2 gemäß EN ISO 15004-2:2007. Im Arbeitsabstand wird kein Gruppe 1 Grenzwert für die vorderen Augenmedien überschritten, allerdings wird der Gruppe 1 Grenzwert betreffend photochemischer Netzhautgefahr $L_{A\text{-}R}$ für das aphake Auge überschritten. Die maximale Expositionsdauer im Arbeitsabstand für ein aphakes Auge (fehlende Linse) beträgt 38,5 Minuten.

Dieser Bericht umfasst die Seiten 1 bis 7

Zeichnungsberechtigter:

Dr. Karl Schulmeister

Sachbearbeiter:

DI Marko Weber

Datum: 09.08.2010

Hinweis:
Das Prüfergebnis bezieht sich ausschließlich auf den Prüfgegenstand.
Ohne schriftliche Genehmigung der Prüfstelle darf der Bericht nicht auszugsweise vervielfältigt werden.

Seibersdorf Labor GmbH | 2444 Seibersdorf, Austria | Tel.: +43 (0) 50550-2500 | Fax: +43 (0) 50550-2502 | Mail: office@seibersdorf-laboratories.at
www.seibersdorf-laboratories.at | Landesgericht Wiener Neustadt | FN 319187v | DVR: 4000728 | UID: ATU64767504 | Steuernummer: 192/6571 | Zertifiziert nach ISO 9001:2000
Bankverbindung: Erste Bank der Österreichischen Sparkassen AG | BLZ: 20111 | Konto Nr.: 291-140-380/00 | IBAN: AT112011129114038000 | BIC: GIBAATWW

10 Annex I

8.1 Registration form for the local ethics commission

Antrag

Version 6.1 vom 03.05.2010 **Bitte immer die *aktuelle* Version verwenden (*http://ethikkommissionen.at*)!**

Dieses Formular soll für Einreichungen bei österreichischen Ethikkommissionen verwendet werden.
Es setzt sich aus einem allgemeinen Teil A - Angaben zur Studie und zum Sponsor -
und aus einem speziellen Teil B - Angaben zu der/den einzelnen Prüfstelle(n) - zusammen.
Bei Einreichungen für mehrere Zentren (Prüfer/innen) muss nur der Teil B an das jeweilige Zentrum angepasst werden.

Adresse der Ethikkommission (optional)	Raum für Eingangsstempel, EK-Nummer, etc. Bitte Freilassen!
Universität Graz LKH-Universitätsklinikum-Eingangsgebäude Auenbruggerplatz 2, 3.OG A-8036 Graz	

ANTRAG AUF BEURTEILUNG EINES KLINISCHEN FORSCHUNGSPROJEKTES

für folgende Prüfer/innen bei folgenden österreichischen Ethikkommissionen:

☒ *Bitte **alle** Ethikkommissionen eintragen, an die der Antrag gesendet wird (**Kurzbezeichnung!**)* ☒
☒ *Im Falle einer **multizentrischen Arzneimittelstudie** ist die **Leitethikkommission** als erste anzuführen!* ☒

Zuständige Ethikkommission	Prüfer/in (lokale Studienleitung)
Medizinische Universität Graz	**Dr.in med.univ. Jutta Horwath-Winter**

Teil A

1. Allgemeines:

1.1 Projekttitel: **Evaluation of a new device to assess the tear film non-invasively**
1.2 Protokollnummer/-bezeichnung: 1.2.1 EudraCT-Nr.:
1.3 Datum des Protokolls: 1.3.1 ISRCTN-Nr.:
1.4 Daten der beiliegenden Amendments: 1.4.1 Nr. 1.4.2 Datum:
1.4.3 Nr. 1.4.4 Datum:
1.4.5 Nr. 1.4.6 Datum:
1.5 Sponsor / Rechnungsempfänger/in (Kontaktperson in der Buchhaltung):

	Sponsor	Rechnungsempfänger/in
1.5.1 Name:	**FH OÖ Forschungs & Entwicklungs GmbH**	
1.5.2 Adresse:	**Franz-Fritsch-Straße 11 / TOP 3 4600 Wels**	
1.5.3 Kontaktperson:	**Dr. Thomas Haslwanter**	
1.5.4 Telefon:	**0732 2008 2170**	
1.5.5 FAX:		
1.5.6 e-mail:	**thomas.haslwanter@fh-linz.at**	
1.5.7 UID-Nummer		**ATU 57300236** (wenn nicht gleich wie „Sponsor“)

2. Eckdaten der Studie

2.1 Art des Projektes:

- ☐ 2.1.1 Klinische Prüfung eines nicht registrierten **Arzneimittels**
- ☐ 2.1.2 Klinische Prüfung eines registrierten **Arzneimittels**
  - ☐ 2.1.2.1 gemäß der Indikation ☐ 2.1.2.2 nicht gemäß der Indikation
- ☐ 2.1.3 Klinische Prüfung einer neuen **medizinischen Methode**
- ☒ 2.1.4 Klinische Prüfung eines **Medizinproduktes**
  - ☐ 2.1.4.1 mit CE-Kennzeichnung ☒ 2.1.4.2 ohne CE-Kennzeichnung
  - ☐ 2.1.4.3 Leistungsbewertungsprüfung (In-vitro-Diagnostika)
- ☐ 2.1.5 **Nicht-therapeutische biomedizinische Forschung** am Menschen (Grundlagenforschung)
- ☐ 2.1.6 **Genetische Untersuchung**
- ☐ 2.1.7 **Sonstiges** (z.B. Diätetik, Epidemiologie, etc.), bitte spezifizieren:

Zusatzinformation: ☒ 2.1.8 **Dissertation** ☐ 2.1.9 **Diplomarbeit**

2.2 Fachgebiet: **Ophthalmologie**

2.3 Arzneimittelstudie (wenn zutreffend)

2.3.1 Prüfsubstanz(en):

2.3.2 Referenzsubstanz:

2.4 Medizinproduktestudie (wenn zutreffend)

2.4.1 Prüfprodukt(e): **Tränenfilmanalysegerät**

2.4.2 Referenzprodukt:

2.5 Klinische Phase: **II** (unbedingt angeben, bei Medizinprodukten die am ehesten zutreffende Phase)

2.6 Nehmen andere Zentren an der Studie teil: ☐ ja ☒ nein. Wenn **ja:**
2.6.1 im Inland ☐ 2.6.2 im Ausland ☐

2.7 Liste der Zentren:

2.8 Liegen bereits Voten anderer Ethikkommissionen vor?
☒ ja ☐ nein. Wenn **ja**, **Voten beilegen!**

2.9 Geplante Anzahl der Prüfungsteilnehmer/innen gesamt (alle teilnehmenden Zentren): **60**

2.10 Charakterisierung der Prüfungsteilnehmer/innen: 2.10.1 Mindestalter: **20** 2.10.2 Höchstalter: **70**
2.10.3 Sind auch nicht persönlich Einwilligungsfähige einschließbar? ☐ ja ☒ nein
2.10.4 Einschließbar sind ☒ weibliche (und/oder) ☒ männliche Teilnehmer/innen.
2.10.5 Sind gebärfähige Frauen einschließbar? ☒ ja ☐ nein

2.11 Dauer der Teilnahme der einzelnen Prüfungsteilnehmer/innen an der Studie: **23 min**
2.11.1 Aktive Phase: 2.11.2 Nachkontrollen:

2.12 Voraussichtliche Gesamtdauer der Studie: **4 Wochen**

3a. Betrifft nur Studien gemäß AMG: Angaben zur Prüfsubstanz (falls nicht in Österreich registriert):

3.1 Registrierung in anderen Staaten? ☐ ja ☐ nein. Wenn **ja**, geben Sie an, in welchen:

3.2 Liegen über das zu prüfende Arzneimittel bereits aussagekräftige Ergebnisse von klinischen Prüfungen vor? ☐ ja ☐ nein

Wenn **ja**, bitte geben Sie folgende Daten an:

3.2.1 In welchen Staaten wurden die Prüfungen durchgeführt:

3.2.2 Phase: ____ (Wenn Studien in mehreren Phasen angeführt sind, die höchste Phase angeben)

3.2.3 Zeitraum:

3.2.4 Anwendungsart(en):

3.2.5 Wurde(n) die klinische(n) Prüfung(en) gemäß GCP-Richtlinien durchgeführt? ☐ ja ☐ nein

3.2.6 Liegt ein Abschlußbericht vor? ☐ ja ☐ nein

Wenn **ja**, bitte legen Sie die **Investigator´s Brochure, relevante Daten** oder ein **Gutachten des Arzneimittelbeirates** bei.

3b. Sonstige im Rahmen der Studie verabreichte Medikamente, deren Wirksamkeit und/oder Sicherheit nicht Gegenstand der Prüfung sind:

Generic Name	Darreichungsform	Dosis

---

4. Betrifft nur Studien gemäß MPG: Angaben zum Medizinprodukt:

4.1 Bezeichnung des Produktes: **Tränenfilmanalysegerät**

4.2 Hersteller: **FH OÖ Forschungs & Entwicklungs GmbH**

4.3 Zertifiziert für diese Indikation: ☐ ja ☒ nein

4.4 Zertifiziert, aber für eine andere Indikation: ☐ ja ☒ nein

4.5 Das Medizinprodukt trägt ein CE-Zeichen ☐ ja ☒ nein

4.6 ☐ Die Produktbroschüre liegt bei.

4.7 Welche Bestimmungen bzw. Normen sind für die Konstruktion und Prüfung des Medizinproduktes herangezogen worden (Technische Sicherheit):

- EN 60601-1 „Medizinische elektrische Geräte - Teil 1: Allgemeine Festlegungen für die Sicherheit einschließlich der wesentlichen Leistungsmerkmale“
- EN ISO 14971 "Anwendung des Risikomanagements auf Medizinprodukte"
- EN ISO 15004 "Ophthalmische Instrumente - Grundlegende Anforderungen und Prüfverfahren - Teil 2: Schutz gegen Gefährdung durch Licht"
- MPG

4.8 Allfällige Abweichungen von den o.a. Bestimmungen (Normen):

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5. Angaben zur Versicherung (gemäß §32 Abs.1 Z.11 und Z.12 und Abs.2 AMG; §§47 und 48 MPG)

5.1 Eine Versicherung ist erforderlich: ☒ ja ☐ nein. Wenn **ja**:

5.1.1 Versicherungsgesellschaft **Zürich Kosmos - wird bei positivem Votum nachgereicht**

5.1.2 Adresse:

5.1.3 Telefon:

5.1.4 Polizzennummer:

5.1.5 Gültigkeitsdauer:

Diese Angaben müssen in der Patienten- / Probandeninformation enthalten sein!

6. Angaben zur durchzuführenden Therapie und Diagnostik

6.1. Welche Maßnahmen bzw. Behandlungen werden **ausschließlich studienbezogen** durchgeführt?

Art	Anzahl/Dosis	Zeitraum	Insgesamt
Tränenfilmanalysegerät (Messung nur an einem Auge)	**1**	**4 min**	**4 min**
Fragebogen (Ocular Surface Disease Index)	**1**	**10 min**	**10 min**
Invasive Tränenfilmaufrisszeit (Messung nur an einem Auge)	**1**	**2 min**	**2 min**
Schirmer I Test (Messung nur an einem Auge)	**1**	**5 min**	**5 min**
Osmolaritätsmessung mittels TearLab (Messung nur an einem Auge)	**1**	**2 min**	**2 min**

6.2. Welche speziellen Untersuchungen (**nur invasive und strahlenbelastende**) werden während des Studienzeitraumes **zu Routinezwecken** durchgeführt:

Art	Anzahl/Dosis	Zeitraum	Insgesamt

6.3. Ergänzende Informationen zu studienbezogenen Maßnahmen und alle erforderlichen Abweichungen von der Routinebehandlung:

Eine Abweichung von der invasiven Routinebehandlung (siehe 7.2) ist die Tatsache, dass die Untersuchung durch die neue Methode berührungslos stattfindet.

>>>>>>>>>>>>>>>>>>>>>>> **BEISPIELE als AUSFÜLLHILFE** <<<<<<<<<<<<<<<<<<<<<<<<<

Art	**Anzahl/Dosis**	**Zeitraum**	**insgesamt**
Blutabnahmen	5ml /jeden 3. Tag	3 Wochen	35ml
Magenbiopsie	1x zu Beginn der Studie	---	1
psychologische Tests	1x zu Beginn; 1x zum Abschluss	3 Wochen	2
Insulin s.c.	2 x 40 IE	1 Woche	2
Thoraxröntgen	1x zu Beginn der Studie	---	1

7. Strukturierte Kurzfassung des Projektes (*in deutscher Sprache, kein Verweis auf das Protokoll*)

7.1 Wenn Original-Projekttitel nicht in Deutsch: Deutsche Übersetzung des Titels: Klinische Prüfung eines neuartigen Gerätes zur berührungslosen Beurteilung der Tränenfilmstabilität
7.2 Zusammenfassung des Projektes (Rechtfertigung, Relevanz, Design, Maßnahmen und Vorgehensweise): Die Erkrankung des "Trockenen Auges" steht in unmittelbarem Zusammenhang mit der Stabilität des Tränenfilms. Ist dieser instabil, befindet sich das Auge permanent in direktem Kontakt mit der Umwelt wodurch Entzündungen und teils massive Befindungsstörungen entstehen. Das Krankheitsbild des „Trockenen Auges“ betrifft laut Studien circa 20% der erwachsenen Bevölkerung, allein in Österreich wird die Zahl der Betroffenen auf 1,0 bis 1,5 Millionen Patienten geschätzt. Ebenfalls essentiell ist die Beurteilung des Tränenfilms im Zuge von Kontaktlinsenanpassungen um in weiterer Folge verschiedene Linsenmaterialien optimal auf den Patienten abzustimmen. Die Bewertung der Tränenflüssigkeit erfolgt bis jetzt invasiv d.h. ein Farbstoff wird auf das Auge aufgebracht und mittels spezieller Beleuchtung der Tränenfilm beurteilt. Diese Methoden sind aufgrund der Einfachheit weit verbreitet. Die Ergebnisse sind allerdings ungenau und subjektiv (3-malige Messung mit einer Stoppuhr). Das entwickelte Medizinprodukt ist ein transportables Produkt der Risikoklasse I. Es erlaubt eingeschultem Fachpersonal Videosequenzen des Augenvordergrundes zu erzeugen. Die Auswertung der Videosequenzen erfolgt NICHT durch das Gerät. Kontraindikation für die Verwendung des Gerätes stellt eine hohe Lichtsensibilität des Patienten dar. Solch eine erhöhte Lichtsensibilität kann bei folgenden Patientengruppen vorkommen und wird als Kontraindikation angesehen: [1] Hornhautentzündungen [2] Akut auftretende Gefäßhautentzündungen [3] Infektiöse Bindehautentzündungen. Das Gerät ist dazu bestimmt ausschließlich in trockenen Räumen angewendet zu werden, die Untersuchung darf nur von eingeschultem Fachpersonal (Ophthalmologen, Optometristen und Untersuchungsassistenten) durchgeführt werden. Im Rahmen der Untersuchung kommt der Patient direkt in Oberflächenkontakt (Hautkontakt im Bereich rund um das Auge) mit elektrisch isolierten Teilen des Gerätes sowie mit der vom Gerät abgegebenen Lichtenergie. Das Auge wird während der Untersuchung nicht berührt. Die Beleuchtungsstärke wird vom Arzt geregelt, das Auge wird vorübergehend (Definition laut RL 93/42/EWG, Anhang IX) während einer Untersuchung für circa vier Minuten bestrahlt. Die sicherheitstechnische Überprüfung der Beleuchtungsstärke wurde von einer akkreditierten Stelle (gemäß EN ISO 15004-2: Ophthalmische Instrumente- Grundlegende Anforderungen und Prüfverfahren Teil 2: Schutz gegen Gefährdung durch Licht) durchgeführt. Das Ergebnis liegt als Anhang bei (Amendment LE-L-0185-1/10).
7.3 Ergebnisse der prä-klinischen Tests oder Begründung für den Verzicht auf prä-klinischen Tests: Der Verzicht auf präklinische Test begründet sich durch ein fehlendes Modell des Auges bzw. des Tränenfilms.
7.4 Primäre Hypothese der Studie (wenn relevant auch sekundäre Hypothesen): Die physikalischen Parameter des Tränenfilms korrelieren mit dem subjektiven Empfinden und den objektiven Tränenfilmparametern des Patienten.
7.5 Relevante Ein- und Ausschlusskriterien: Im Zuge der Studie sollen PatientInnen mit leichten, mäßigen und schweren Beschwerden untersucht werden. Als mögliches Ausschlusskriterium kann eine erhöhte Lichtempfindlichkeit angesehen werden, beispielsweise bei Hornhautentzündungen, akut auftretenden Gefäßhautentzündungen oder infektiösen Bindehautentzündungen.
7.6 Ethische Überlegungen (Identifizieren und beschreiben Sie alle möglicherweise auftretenden Probleme. Beschreiben Sie den möglichen Wissenszuwachs, der durch die Studie erzielt werden soll und seine Bedeutung, sowie mögliche Risiken für Schädigungen oderBelastungen der Prüfungsteilnehmer/innen. **Legen Sie Ihre eigene Bewertung des Nutzen/Risiko-Verhältnisses dar**): Ein möglicherweise auftretendes Problem kann die Lichteinwirkung auf das Auge sein. An dieser

	Stelle sei jedoch anzumerken, dass das Gerät von einer staatlich akkreditierten Prüfungsstelle als harmlos eingestuft wurde. Erst ab einer Anwendungsdauer von ca. 40 min wird es für den Patienten gefährlich. Da der normale Untersuchungsablauf nicht mehr als 4 Minuten dauert befindet sich das Gerät weit unter diesen Grenzwerten. Da keine berührbaren metallischen Teile am Gerät vorhanden sind gibt es praktisch keine Gefährdung durch elektrischen Strom für den Patienten. Dieser Punkt, zusammen mit der Nichtinvasivität und der oben beschriebenen Harmlosigkeit gegenüber Lichtbestrahlung, begründen das geringe Risiko (Risikoklasse I). Der medizinische Nutzen ist dadurch gegeben, dass man die Tränenfilmstabilität beurteilen kann ohne das Auge berühren zu müssen.
7.7	Begründung für den Einschluss von Personen aus geschützten Gruppen (z.B. Minderjährige, temporär oder permanent nicht-einwilligungsfähige Personen; wenn zutreffend): Die Patienten werden nicht aus geschützten Gruppen rekrutiert.
7.8	Beschreibung des Rekrutierungsverfahrens (alle zur Verwendung bestimmte Materialien, z.B. Inserate **inkl. Layout** müssen beigelegt werden): Da die Krankheit des "Trockenen Auges" eine sehr hohe Inzidenzrate aufweist, müssen keine Inserate geschalten werden. Die Patienten werden im Zuge des Klinikalltags bzw. im Kollegen - bzw. Bekanntenkreis rekrutiert.
7.9	Vorgehensweise an der/den Prüfstelle(n) zur Information und Erlangung der informierten Einwilligung von Prüfungsteilnehmer/inne/n, bzw. Eltern oder gesetzlichen Vertreter/inne/n, wenn zutreffend (wer wird informieren und wann, Erfordernis für gesetzliche Vertretung, Zeugen, etc.): Der Patient wird unmittelbar vor der Untersuchung aufgeklärt und unterschreibt die Einwilligung vor Ort.
7.10	Risikoabschätzung, vorhersehbare Risiken der Behandlung und sonstiger Verfahren, die verwendet werden sollen (inkl. Schmerzen, Unannehmlichkeiten, Verletzung der persönlichen Integrität und Maßnahmen zur Vermeidung und/oder Versorgung von unvorhergesehenen / unerwünschten Ereignissen): Als Unannehmlichkeit kann die Lichtabstrahlung des Gerätes aufgefasst werden. Ein bleibender Schaden kann jedoch ausgeschlossen werden, da das Gerät gem. EN ISO 15004 geprüft wurde und als sicher eingestuft werden kann (siehe Prüfbericht) .
7.11	Voraussichtliche Vorteile für die eingeschlossenen Prüfungsteilnehmer/innen: Die Stabilität des Tränenfilms kann berührungslos bestimmt werden.
7.12	Relation zwischen Prüfungsteilnehmer/in und Prüfer/in (z.B. Patient/in - Ärztin/Arzt, Student/in - Lehrer/in, Dienstnehmer/in - Dienstgeber/in, etc.): Patient/in - Ärztin/Arzt
7.13	Verfahren an der/den Prüfstelle(n) zur Feststellung, ob eine einzuschließende Person gleichzeitig an einer anderen Studie teilnimmt oder ob eine erforderliche Zeitspanne seit einer Teilnahme an einer anderen Studie verstrichen ist (von besonderer Bedeutung, wenn gesunde Proband/inn/en in pharmakologische Studien eingeschlossen werden): Die Teilnahme an anderen Studien stellt kein Ausschlusskriterium dar.
7.14	Methoden, um unerwünschte Effekte ausfindig zu machen, sie aufzuzeichnen und zu berichten (Beschreiben Sie wann, von wem und wie, z.B. freies Befragen und/oder an Hand von Listen): Der Patient wird unmittelbar nach der Untersuchung befragt. Der Untersucher führt ein Protokoll mit um etwaige Ereignisse dokumentieren zu können.
7.15	Optional: Statistische Überlegungen und Gründe für die Anzahl der Personen, die in die Studie eingeschlossen werden sollen (ergänzende Informationen zu Punkt 8, wenn erforderlich):
7.16	Optional: Verwendete Verfahren zum Schutz der Vertraulichkeit der erhobenen Daten, der Quelldokumente und von Proben (ergänzende Informationen zu Punkt 8, wenn erforderlich):
7.17	Plan zur Behandlung oder Versorgung nachdem die Personen ihre Teilnahme an der Studie beendet haben (wer wird verantwortlich sein und wo):

Die unmittelbare Versorgung übernimmt der/die klinische Prüfer/in.
7.18 Betrag und Verfahren der Entschädigung oder Vergütung an die Prüfungsteilnehmer/innen (Beschreibung des Betrages, der während der Prüfungsteilnahme bezahlt wird und wofür, z.B. Fahrtspesen, Einkommensverlust, Schmerzen und Unannehmlichkeiten, etc.): Durch den überschaubaren Aufwand seitens des Patienten ist keine Vergütung geplant.
7.19 Regeln für das Aussetzen oder vorzeitige Beenden der Studie an der/den Prüfstelle(n) in diesem Mitgliedstaat oder der gesamten Studie: Sollte in einer Situation eine Gefährdung des Patienten auftreten wird die Studie sofort abgebrochen.
7.20 Vereinbarung über den Zugriff der Prüferin/des Prüfers/der Prüfer auf Daten, Publikationsrichtlinien, etc. (wenn nicht im Protokoll dargestellt): siehe "Handbuch des klinischen Prüfers"
7.21 Finanzierung der Studie (wenn nicht im Protokoll dargestellt) und Informationen über finanzielle oder andere Interessen der Prüferin/des Prüfers/der Prüfer: Es sind keinerlei finanzielle Interessen seitens des/der klinischen Prüfer/in vorhanden.
7.22 Weitere Informationen (wenn erforderlich):

8. Biometrie, Datenschutz: ***!!! Achtung: Pkt. 8.1 ist in jedem Fall auszufüllen !!!***

(Hier nur Kurzinformationen in Stichworten, ausführlicher - wenn erforderlich - unter Punkt 7.15 und 7.16)

8.1 Studiendesign (z.B. doppelblind, randomisiert, kontrolliert, Placebo, Parallelgruppen, multizentrisch)

☐ 8.1.1 offen	☐ 8.1.2 randomisiert	☐ 8.1.3 Parallelgruppen	☐ 8.1.4 monozentrisch
☐ 8.1.5 blind	☐ 8.1.6 kontrolliert	☐ 8.1.7 cross-over	☐ 8.1.8 multizentrisch
☐ 8.1.9 doppelblind	☐ 8.1.10 Placebo	☐ 8.1.11 faktoriell	☒ 8.1.12 Pilotprojekt
☐ 8.1.13 observer-blinded	☐ 8.1.14 Äquivalenzprüfung		
☐ 8.1.15 sonstiges:			

8.1.16 Anzahl der Gruppen: **1**

8.1.17 Stratifizierung: ☒ nein ☐ ja: Kriterien:

8.1.18 Messwiederholungen: ☒ nein ☐ ja: Zeitpunkte:

8.1.19 Hauptzielgröße: **Nichtinvasive Tränenfilmaufrisszeit**

8.1.20 Nullhypothese(n): **Die physikalischen Parameter des Tränenfilms korrelieren nicht mit dem subjektiven Empfinden und den objektiven Tränenfilmparametern des Patienten.**

8.1.21 Alternativhypothese(n): **Die physikalischen Parameter des Tränenfilms korrelieren mit dem subjektiven Empfinden und den objektiven Tränenfilmparametern des Patienten.**

8.1.22 Nebenzielgrößen: **Nichtinvasive Tränenfilmstabilisationszeit, Ausbreitungsrichtung des Lipidfilms, Flächenwachstum der tränenfilmfreien Oberfläche, Lokalisation und Muster der Austrocknung**

8.2 Studienplanung

Die Fallzahlberechnung basiert auf (Alpha = Fehler 1. Art, Power = 1 – Beta = 1 – Fehler 2. Art):

8.2.1 Alpha: 8.2.2 Power: 8.2.3 Stat.Verfahren:

8.2.4 Multiples Testen: ☐ nein ☐ ja: Korrekturverfahren.:

8.2.5 Erwartete Anzahl von Studienabbrecher/inne/n (Drop-out-Quote):

8.3 Geplante statistische Analyse

Population: ☐ 8.3.1 Intention-to-treat ☐ 8.3.2 Per protocol

8.3.3 Zwischenauswertung: ☐ nein ☐ ja: Abbruchkriterien:

8.3.4 Geplante statistische Verfahren: **Lineare Regressionsanalyse**

8.4 Dokumentationsbögen / Datenmanagement

8.4.1 Angaben zur Datenqualitätsprüfung

8.4.2 Angaben zum Datenmanagement

Die Daten werden lokal auf dem Rechner abgespeichert und gesammelt und anonym analysiert.

8.5 Verantwortliche und Qualifikation

8.5.1 Wer führte die biometrische Planung durch (ggf. Nachweis der Qualifikation)?

Dr. Thomas Haslwanter

8.5.2 Wer wird die statistische Auswertung durchführen (ggf. Nachweis der Qualifikation)?

Dr. Thomas Haslwanter

8.6 Datenschutz

8.6.1 Die Datenverarbeitung erfolgt a) ☐ personenbezogen b) ☒ indirekt personenbezogen

8.6.2 Wenn a): Begründung:

DVR-Nummer:

8.6.3 Wenn b): Wie erfolgt die Anonymisierung?

Nummerierung d. Probanden, Die Liste mit den Namen und den zugehörigen Nummern bleibt beim Prüfungsleiter.

9. Liste der eingereichten Unterlagen (wenn nicht gesondert dem Antrag beiliegend)**:**

Dokument	Version/Identifikation	Datum
Protokoll	**V 1.1**	**30.05.11**
Kurzfassung	**im Antrag integriert**	
Patienteninformation / Einwilligungserklärung	**V 1.1**	**20.05.11**
Prüfbogen (Case Report Form, CRF)	**V 1.1**	**30.05.11**
Versicherungsbestätigung	**wird bei pos. Bescheid nachgereicht**	
Amendment Nr.		
Amendment Nr.		
Amendment Nr.		
Lokales Amendment Nr.		
Prüfbericht LE-L-0185-1/10 gem. EN ISO 15004 - 2:2007		**09.08.10**
Risikomanagementakte Tränenfilmanalysegerät	**V 1.3**	**07.01.10**
EMV- Teilprüfbericht Elektromagnetische Verträglichkeit EMVC 2011-03-13		**28.03.11**

Name und Unterschrift der Antragstellerin/des Antragstellers

9.1 Name: **Dr. Thomas Haslwanter**

9.2 Institution/ Firma: **FH OÖ Forschungs-und Entwicklungs Gmbh**

9.3 Position: **Projektleiter "Dry Eye"**

9.4 Antragsteller/in ist (nur AMG-Studien)

- ☐ 9.4.1 koordinierende/r Prüfer/in (multizentrische Studie)
- ☐ 9.4.2 Hauptprüfer/in (monozentrische Studie)
- ☐ 9.4.3 Sponsor bzw. Vertreter/in des Sponsors
- ☐ 9.4.4 vom Sponsor autorisierte Person/Organisation

Ich bestätige hiermit, dass die in diesem Antrag gemachten Angaben korrekt sind und dass ich der Meinung bin, dass die Durchführung der Studie in Übereinstimmung mit dem Protokoll, nationalen Regelungen und mit den Prinzipien der Guten Klinischen Praxis möglich sein wird.

Weiters stimme ich mit meiner Unterschrift zu, dass folgende Daten aus meinem Antrag ggf. durch die Ethikkommission veröffentlicht werden, um die Anträge nach Zahl und Inhalt transparent zu machen: EK-Nummer, Einreich-Datum, Projekttitel, Hauptprüfer, Sponsor/CRO, weitere Zentren.
(Im Falle der Nicht-Zustimmung bitte diesen Absatz durchzustreichen)

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Unterschrift der Antragstellerin/des Antragstellers Datum

!!! Achtung: Diese Unterschrift ist in jedem Fall erforderlich !!!

Teil B

Studienkurzbezeichnung: **Klinische Prüfung eines neuartigen Gerätes zur Ermittlung der Tränenfilmstabilität**

10. Angaben zur Prüferin/zum Prüfer

10.1 Name: **Dr.in med.univ. Jutta Horwath-Winter**

10.2 Krankenanstalt/Institut/Abteilung: **Universitäts-Augenklinik Graz, Augenheilkunde**

10.3 Telefon	10.4 „Pieps"/Mobil	10.5 Fax	10.6 e-mail-Adresse:
0316 385 80807			

10.7 Jus practicandi: ☐ ja ☒ nein 10.8 Facharzt für: **Augenheilkunde und Optometrie**

10.9 Prüfärztekurs: ☐ ja ☒ nein

10.10 Sofern relevant: Präklinische Qualifikation (z.B. Labordiagnostik) bzw. Name der Verantwortlichen:

11. Geplante Anzahl der Patient/inn/en bzw. Proband/inn/en an dieser Prüfstelle 60

12. Verantwortliche Mitarbeiter/innen an der klinischen Studie (an Ihrer Prüfstelle)

Fr/Hr	Titel	Vorname	Name	Institution
Fr	PD Dr	Ingrid	Boldin	Universitäts-Augenklinik Graz
Hr	Dr	Dieter	Rabensteiner	Universitäts-Augenklinik Graz

13. Unterschrift der Prüferin/des Prüfers

Ich bestätige hiermit, dass die in diesem Antrag gemachten Angaben korrekt sind und dass ich der Meinung bin, dass die Durchführung der Studie in Übereinstimmung mit dem Protokoll, nationalen Regelungen und mit den Prinzipien der Guten Klinischen Praxis möglich sein wird.

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Unterschrift der Prüferin/des Prüfers Datum

Bei multizentrischen AMG-Studien sind die Teile B von der Hauptprüferin/dem Hauptprüfer des jeweiligen Zentrums zu unterzeichen. Alternativ zur Unterschrift auf den Teilen B können die Unterschriften der Hauptprüfer/innen auch auf den Unterschriftenseiten des Protokolls oder der Prüfärzteverträge vorgelegt werden. Es muss jedenfalls eine eindeutige - durch Unterschrift dokumentierte - Zustimmung aller Hauptprüfer/innen zum Protokoll vorliegen.

14. Name und Unterschrift der Leiterin/des Leiters ☐ der Organisationseinheit ☐ des Pflegedienstes*

14.1 Name:

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Unterschrift der Leiterin/des Leiters Datum

** Die Unterschrift der Leiterin/des Leiters des Pflegedienstes ist für Pflegeforschungsprojekte und die Anwendung neuer Pflegekonzepte und -methoden erforderlich, ansonsten die Unterschrift der Leiterin/des Leiters der jeweiligen Organisationseinheit.*
Organisationseinheit: die Klinik (wenn gegliedert: die klinische Abteilung), die Abteilung oder die gemeinsame Einrichtung

!!! Achtung: Teil B ist in jedem Fall vollständig auszufüllen, bei multizentrischen klinischen Prüfungen nach AMG für jedes in Österreich teilnehmende Zentrum separat !!!

Registration form for the AGES

Bundesamt für Sicherheit im Gesundheitswesen

AGES PharmMed
Institut Inspektionen, Medizinprodukte & Hämovigilanz
Schnirchgasse 9, 1030 Wien

Meldung einer klinischen Prüfung eines Medizinproduktes (MP)

gemäß § 40 Medizinproduktegesetz (MPG), BGBl. Nr. 657/1996 idgF

An das
Bundesamt für Sicherheit im Gesundheitswesen
Institut Inspektionen, Medizinprodukte & Hämovigilanz
Schnirchgasse 9
1030 Wien

I. Allgemeine Angaben

Identifizierung der Studie: *Akademische Studie- Evaluation of a new device to assess the tear-film non-invasively*

1. Titel/Kurztitel und Versionsbezeichnung (Nummer und/oder Datierung) des klinischen Prüfplans:
   Klinischer Prüfplan Tränenfilmanalysegerät gemäß EN ISO 14155-2, V1.1, Erstellungsdatum: 20.05.2011

2. Name/Bezeichnung des geprüften Medizinproduktes (zur Identifizierung notwendige Daten):
   Behandlungseinheit, bestehend aus:
   - *Notebook: ACER Travelmate 4651LMI 15f , Seriennummer: GK 2005-208, Betriebssystem: WIN XP, SP 3, Aufnahmesoftware: V 1.0*
   - *Trenntrafo: Trenntransformator ERT230/230/4/G, Thalheimer Transformatorenwerke GmbH, Seriennummer: DE 31732856*
   - *Tränenfilmanalysegerät, Eigenproduktion der FH OÖ Forschungs-und Entwicklungs GmbH*

3. Art/Beschreibung des geprüften Medizinproduktes:
   Das entwickelte Medizinprodukt ist ein transportables Produkt der Risikoklasse I. Es erlaubt eingeschultem Fachpersonal Videosequenzen des Augenvordergrundes zu machen. Die Bewertung der Videos erfolgt NICHT durch das Gerät.

4. Einstufung/Klassifizierung des geprüften Medizinprodukts:
   - ☐ Aktives implantierbares medizinisches Gerät (AIMD) = Produkt gem. RL 90/385/EWG
   - ☒ Medizinprodukt nach Risikoklasse = Produkt gem. RL 93/42/EWG
     - ☒ Klasse I gem. Regel *10,12* (Anh. IX, RL 93/42/EWG)
     - ☐ Klasse IIa gem. Regel (Anh. IX, RL 93/42/EWG)
       - ☐ invasiv zur langzeitigen Anwendung bestimmt
     - ☐ Klasse IIb gem. Regel (Anh. IX, RL 93/42/EWG)
       - ☐ invasiv zur langzeitigen Anwendung bestimmt
     - ☐ Klasse III gem. Regel (Anh. IX, RL 93/42/EWG)
   - ☐ Produkt ist ein Implantat
   - ☐ Medizinprodukt hat eine CE-Kennzeichnung
     - ☐ Anwendung erfolgt **in** der vom Hersteller ausgewiesenen Zweckbestimmung
     - ☐ Anwendung erfolgt **außerhalb** der vom Hersteller ausgewiesenen Zweckbestimmung
   - ☐ Medizinprodukt unter Verwendung von Gewebe tierischen Ursprungs hergestellt (siehe RL 2003/32/EG)
   - ☐ Medizinprodukt enthält Komponente(n) aus menschlichem Blut oder Blutplasma (siehe RL 2000/70/EG bzw. RL 2001/104/EG)

Meldung einer klinischen Prüfung eines Medizinproduktes (MP)

gemäß § 40 Medizinproduktegesetz (MPG), BGBl. Nr. 657/1996 idgF

Identifizierung der Studie: *Akademische Studie- Evaluation of a new device to assess the tear-film non-invasively*

5. Nähere Angaben zum geprüften Medizinprodukt: Vorgesehene(r) Anwendungsbereich(e) des Medizinproduktes; Hauptindikation(en), Art der Anwendung:

Das entwickelte Medizinprodukt ist ein transportables Produkt der Risikoklasse I. Es erlaubt eingeschultem Fachpersonal Videosequenzen des Augenvordergrundes zu machen. Die Bewertung der Videos erfolgt NICHT durch das Gerät.

Klinische Indikation für die Verwendung dieses Gerätes ist das Krankheitsbild des „Trockenen Auges", ein Leiden, das unmittelbar auf einen instabilen Tränenfilm zurückzuführen ist. Kontraindikationen für die Verwendung des Gerätes stellt eine hohe Lichtsensibilität des Patienten dar. Solch eine erhöhte Lichtsensibilität kommt bei folgenden Patientengruppen vor und wird als Kontraindikation angesehen:

[1] Hornhautentzündungen

[2] Akut auftretende Gefäßhautentzündungen

[3] Bindehautentzündungen.

Das Gerät ist dazu bestimmt ausschließlich in trockenen Räumen angewendet zu werden, die Untersuchung darf nur von eingeschultem Fachpersonal (Ophthalmologen, Optometristen und Untersuchungsassistenten) durchgeführt werden. Im Rahmen der Untersuchung kommt der Patient direkt in Oberflächenkontakt (im Bereich rund um das Auge) mit elektrisch isolierten Teilen des Gerätes sowie mit vom Gerät abgegebener Lichtenergie. Das Auge wird während der Untersuchung nicht berührt. Die Beleuchtungsstärke wird vom Arzt geregelt, das Auge wird vorübergehend (Definition laut RL 93/42/EWG, Anhang IX) während einer Untersuchung für circa vier Minuten bestrahlt. Die Beleuchtungsstärke wurde von einer akkreditierten Stelle gemäß EN ISO 15004-2: Ophthalmische Instrumente- Grundlegende Anforderungen und Prüfverfahren Teil 2: Schutz gegen Gefährdung durch Licht) bewertet.

6. Sponsor:		7. Repräsentant des Sponsors in Österreich oder im EWR[1]:	
Firma:	*FH OÖ Forschungs-und Entwicklungs GmbH*	Firma:	
Kontaktperson:	*DI(FH) Michael Ring*	Kontaktperson:	
Straße:	*Franz-Fritsch-Straße 11/ TOP 3*	Straße:	
PLZ Ort:	*4600 Wels*	PLZ Ort:	
Land:	*Österreich*	Land:	
Tel.:	*+43 732 2008 5010*	Tel.:	
Fax:		Fax:	
e-mail:	*michael.ring@fh-linz.at*	e-mail:	

[1] Der Sponsor muss in einer Vertragspartei des EWR niedergelassen sein. (siehe MPG § 3 Abs. 5)

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gemäß § 40 Medizinproduktegesetz (MPG), BGBl. Nr. 657/1996 idgF

8. Hersteller:	9. Gebührenvorschreibung ergeht an (falls nicht an den Sponsor):
Firma: *FH OÖ Forschungs-und Entwicklungs GmbH*	Firma:
Kontaktperson: *DI(FH) Michael Ring*	Kontaktperson:
Straße: *Franz-Fritsch-Straße 11/ TOP 3*	Straße:
PLZ Ort: *4600 Wels*	PLZ Ort:
Land: *Österreich*	Land:
Tel.: *+43 732 2008 5010*	Tel.:
Fax:	Fax:
e-mail: *michael.ring@fh-linz.at*	e-mail:
10. Voraussichtlicher **Beginn** der klinischen Prüfung: Monat/Jahr (MM.JJJJ): *09.2011*	11. Voraussichtliches **Ende** der klinischen Prüfung: Monat/Jahr (MM.JJJJ): *10.2011*

Identifizierung der Studie: *Akademische Studie- Evaluation of a new device to assess the tear-film non-invasively*

II. Nähere Angaben zur klinischen Prüfung

12. Die klinische Prüfung wird durchgeführt ☒ stationär ☒ ambulant ☐ im niedergelassenen Bereich

13. Geplante Anzahl der Prüfungsteilnehmer:
in Österreich: *60* im EWR (ohne Österreich): außerhalb EWR:

14. Wird die klinische Prüfung monozentrisch/multizentrisch durchgeführt?
☒ monozentrisch
☐ multizentrisch
Geplante Anzahl der Prüfzentren:
in Österreich: *1* im EWR (ohne Österreich): außerhalb EWR:

15. Liste der Prüfzentren in Österreich (Für jedes angegebene Prüfzentrum ist ein Beiblatt „Prüfzentren"[2] auszufüllen):
- *Universitäts-Augenklinik Graz, Augenheilkunde*
-
-
-
-

[2] Beiblatt „Prüfzentren" siehe www.basg.at/Medizinprodukte/Formulare

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16. In welchen EWR-Staaten (außer Österreich) wird die klinische Prüfung durchgeführt (mit Anzahl der jeweiligen Prüfzentren und Prüfungsteilnehmer):

• Land:	Ort:	Anzahl Zentren:	Anzahl Prüfungsteilnehmer:
• Land:	Ort:	Anzahl Zentren:	Anzahl Prüfungsteilnehmer:
• Land:	Ort:	Anzahl Zentren:	Anzahl Prüfungsteilnehmer:
• Land:	Ort:	Anzahl Zentren:	Anzahl Prüfungsteilnehmer:
• Land:	Ort:	Anzahl Zentren:	Anzahl Prüfungsteilnehmer:

17. Zielsetzung bzw. Zweck der klinischen Prüfung:

Zweck der klinischen Prüfung ist es, den Nutzen des Tränenfilmanalysegeräts den Risiken gegenüberzustellen und klinisch zu bewerten. Ziel ist es demnach, die Qualität und damit verbundene Interpretierbarkeit der Videoaufnahmen zu bestätigen.

Vorliegende Studie findet im Rahmen einer Dissertation statt und ist demnach als akademische Studie anzusehen.

18. Studiendesign (randomisiert, cross-over, parallel, verblindet, doppelblind, kontrolliert, etc.):

Pilotstudie

Identifizierung der Studie: *Akademische Studie- Evaluation of a new device to assess the tear-film non-invasively*

19. Werden Vergleichsgruppen mitgeprüft? ☒ nein ☐ ja

20. Wenn Pkt. 19 beantwortet mit ja, welche Vergleichsgruppen werden mitgeprüft? (bitte jeweils kurz spezifizieren)
- ☐ andere Medizinprodukte:
- ☐ Arzneimittel:
- ☐ sonstige Vergleichsstandards (z.B. Plazebo, keine Behandlung):

21. Liegen über das zu prüfende Medizinprodukt bereits aussagekräftige Ergebnisse von klinischen Prüfungen vor?

☒ nein ☐ ja; Wenn ja, bitte Literaturliste bzw. Literatur beilegen!

22. Relevantes **Zubehör** zum geprüften Medizinprodukt spezifizieren:

keines

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23. In der gegenständlichen Studie zum Funktionieren des Medizinproduktes eingesetzte **Software** spezifizieren:

Zweck der eingesetzten Software ist den Anwender durch den Untersuchungsablauf zu führen und das aufgenommene Video darzustellen. Die Bewertung der Videos erfolgt NICHT durch die Software-Komponente.

24. Enthält das Medizinprodukt Arzneimittelkomponenten in unterstützender Funktion?

☒ nein
☐ ja, welche?

25. Dient das Medizinprodukt der Verabreichung von Arzneimitteln?

☒ nein
☐ ja, welche?

26. Welche Begleitbehandlungen/therapeutische Maßnahmen (Therapieverfahren, Medikation etc.) bzw. diagnostische Tests und Untersuchungen werden ausschließlich studienbedingt durchgeführt?

Tränenfilmanalysegerät (4 Minuten)

Fragebogen: Ocular Surface Disease Index (10 Minuten)

Invasive Tränenfilmaufrisszeit (2 Minuten)

Schirmer I Test (5 Minuten)

Osmolaritätsmessung mittels TearLab (2 Minuten)

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III. Folgende Dokumente sind dieser Meldung beigelegt:

- ☒ klinischer Prüfplan / Protokoll
- ☒ Handbuch des klinischen Prüfers (Investigators Brochure)
- ☐ Gebrauchsanweisung in deutscher Sprache bei Medizinprodukten mit CE-Kennzeichnung
- ☒ Befürwortende Stellungnahme(n) der zuständigen Ethikkommission(en) („positives Votum")
- ☒ Aufklärungsinformation und Einwilligungserklärung für Prüfungsteilnehmer in deutscher Sprache (Informed Consent Form)
- ☒ Bestätigung über den Versicherungsschutz der Prüfungsteilnehmer
- ☒ Schriftliche Zusicherung, dass das Medizinprodukt den grundlegenden Anforderungen der jeweils relevanten Richtlinie entspricht und alle Sicherheitsvorkehrungen für die Prüfungsteilnehmer bzw. Anwender getroffen sind[3]
- ☐ Konformitätserklärung des Herstellers
- ☒ Zertifikat(e) benannter Stellen
- ☐ Unterlagen zur Qualifikation des(r) klinischen Prüfer(s)
- ☐ Vereinbarungen zwischen dem Sponsor, Monitor und klinischem Prüfer, die deren Verantwortlichkeiten festlegen
- ☒ Prüfbogen (CRF, Case Report Form)
- ☐ Unterlagen zur Konstruktion bzw. zur Herstellung (Fertigungsverfahren, Sterilisation etc.)
- ☒ Ergebnisse von Prüfungen oder technischen Tests (z.B. Biokompatibilität[4], elektrische Sicherheit[5], etc.)
- ☒ Ergebnisse der Risikoanalyse
- ☒ Liste der ganz oder teilweise angewandten Normen (zu finden im Handbuch des klinischen Prüfers)
- ☐ Unterlagen zur Sicherheit von Komponente(n) tierischen[6] oder menschlichen[7] Ursprungs

☐ weitere Unterlagen, und zwar:

[3] Grundlegende Anforderungen: siehe RL 90/385/EWG, Anh. I und RL 93/42/EWG, Anh. I
[4] Siehe EN ISO 10993
[5] Siehe Normenreihe EN 60601
[6] Siehe RL 2003/32/EG, MEDDEV Guidelines der Europäischen Kommission
[7] Siehe RL 2000/70/EG bzw. RL 2001/104/EG

Bundesamt für Sicherheit
im Gesundheitswesen

AGES PharmMed
Institut Inspektionen, Medizinprodukte & Hämovigilanz
Schnirchgasse 9, 1030 Wien

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Ab Mai 2011 sind klinische Studien mit Medizinprodukten in die europäische Datenbank Eudamed durch eine nationale Behörde (in Österreich durch das BASG) einzupflegen. Die Eingabe hat durch diejenige nationale Behörde zu erfolgen, bei der die erste Meldung der klinischen Studie eingebracht wurde. (siehe Medizinproduktemeldeverordnung)

Bei welcher nationalen Behörde im EWR erfolgt/erfolgte die erste Meldung der gegenständlichen klinischen Studie:

☒ Österreich ☐ andere Behörde im EWR; falls zutreffend Angabe der Eudamed Nummer:

Ich erkläre, dass alle Angaben korrekt und vollständig sind und akzeptiere die anfallenden Gebühren gemäß der „Verordnung des Bundesamtes für Sicherheit im Gesundheitswesen über den **Gebührentarif** gemäß GESG".
(siehe www.basg.at/ueber-uns/tarife/)

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09.08.2011, MICHAEL RING
Michael

Stempel des Sponsors | Datum und Unterschrift (Name auch in Blockschrift)

Ohne abweichende Zustimmung der Ethikkommission werden ein Studienaufbau und eine Dokumentation nach den Normen EN ISO 14155-1 und -2 vorausgesetzt. Die grundlegenden Anforderungen für Medizinprodukte sind im Anhang I der europäischen Richtlinien 93/42/EWG oder 90/385 EWG beschrieben. Deren Einhaltung wird vermutet, wenn das Medizinprodukt mit anwendbaren europäischen harmonisierten Normen übereinstimmt.

ACHTUNG!
Die klinische Prüfung ist **vor ihrem Beginn** an das Bundesamt für Sicherheit im Gesundheitswesen zu melden.
(siehe § 40 MPG idgF)

Cornea

Introducing a New Parameter for the Assessment of the Tear Film Lipid Layer

Michael H. Ring,[1,2] *Dieter F. Rabensteiner,*[3] *Jutta Horwath-Winter,*[3] *Ingrid Boldin,*[3] *Robert Hörantner,*[3,4] *and Thomas Haslwanter*[1]

Purpose. The differential diagnosis of dry eye syndrome is still a challenging task. The purpose of this study was to understand the relationship between a novel, objective clinical parameter, the "corrected lipid layer stabilization time," and commonly performed clinical tests for dry eye patients.

Methods. Data were obtained from a prospective clinical study with 59 patients of different subjective severity, as determined with the Ocular Surface Disease Index (OSDI). The dynamics of the tear film lipid layer were made visible through a white light source and were stored digitally. Because the distance between the upper and lower eyelid affects the lipid layer dynamics and varies significantly between subjects, the distance of the eyelids was determined and used to correct the lipid layer stabilization time. The resulting parameter was compared with common clinical procedures.

Results. The corrected lipid layer stabilization time has a highly significant correlation with tear film breakup time (Spearman r $= -0.485$, $P < 0.01$), Schirmer test without anesthesia (r $= -0.431$, $P < 0.01$) and with the Ocular Surface Disease Index (r $= 0.498$, $P < 0.01$). It also correlates with the lissamine green staining score (r $= 0.379$, $P < 0.05$), but shows no correlation with the osmolarity of the tear film. Without the correction for the eyelid opening, the correlations decrease considerably.

Conclusions. These data suggest that the diagnostic value of the lipid layer stabilization time for the assessment of the severity of dry eye syndrome increases considerably when it is corrected by the distance of the eyelids. (*Invest Ophthalmol Vis Sci.* 2012;53:000–000) DOI:10.1167/iovs.12-10257

Some of the main purposes of the pre-ocular tear film are the maintenance of a proper homeostasis and the protection of the exposed ocular surface cells. To fulfill this function, the tear film is organized in three different layers: the innermost layer consists mainly of mucins secreted by ocular surface epithelial cells; the middle layer provides the aqueous solution for proteins and electrolytes and is secreted primarily by the lacrimal glands; the outermost layer, consisting mainly of nonpolar and polar lipids, seals the tear film. It thereby prevents the aqueous compartments from hyperevaporation.[1–3] In healthy subjects, the tear film is rebuilt with each blink and remains stable during the interblink interval.

Popular risk factors that harm the construct of the tear film include contact lens wearing,[4] working in air-conditioned environments,[5] visual display terminal usage,[6,7] and the hormonal status.[8] Additionally, Laser-Assisted in situ Keratomileusis (LASIK) surgeries[9] and autoimmune disorders, such as Sjogrens syndrome,[10] ~~are~~ contribute to the destabilization process.

The diagnosis of an insufficient tear film is often referred to as dry eye disease (DED) or keratoconjunctivitis sicca, which can be considered as a subgroup of ocular surface diseases.[11] It is defined as a "multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear instability with potential damage to the ocular surface. Dry eye is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface."[12]

Symptoms of discomfort, dryness, or grittiness soon occur if any of the previously described layers is lacking functionality.[13] Because the tear film is the first refracting structure for the incoming light, an inhomogeneous tear film is also often the reason for transient changes in visual performance after a blink.[14,15]

Due to the diversity of risk factors and etiology, as well as the complex interactions between the layers, the precise diagnosis of DED is challenging. A number of standard assessment procedures for tear film disorders have been developed and can be divided into subgroups: questionnaires to assess the subjectively reported symptoms; investigations of ocular surface damage via staining; the demonstration of the tear film instability by tear film break up time (TFBUT) as determined with fluorescein; and the lacrimal gland production capacity through the Schirmer test.[12] It is remarkable that these clinical investigations correlate quite poorly with the subjectively reported symptoms and vice versa.[13] This can be explained by the highly dynamic characteristics of the tear film and its sensitivity to the surrounding environment, as well as to the often invasively performed diagnostic procedures. Thus, the search for an objective, noninvasive but robust parameter for the diagnosis of DED is still of potential interest for both science and clinical routine. More recently, devices for measuring the tear film osmolarity have also been introduced, for example, Tearlab (Tearlab, Osmolarity System, Ocusense, San Diego, CA).

Besides those commonly performed investigations, a couple of noninvasively recorded parameters have been established to diagnose DED. Mengher et al. were among the first to record the noninvasive tear film break up time (NITBUT) by investigating the distortions of a pattern projected on the tear film.[16,17] Additionally, the tear film meniscus height[18] and the tear meniscus curvature[19] have been reported to give information on the quantity of the tear film. Nevertheless,

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Submitted for publication May 24, 2012; revised August 4 and 18, 2012; accepted August 20, 2012.

Disclosure: M.H. Ring, None; D.F. Rabensteiner, None; J. Horwath-Winter, None; I. Boldin, None; R. Hörantner, None; T. Haslwanter, None

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commercially available devices for the assessment of these parameters are currently not widely distributed.

The importance of the outermost tear film lipid layer for the overall stability of the construct of the tear film was reviewed by Foulks et al.[20] They concluded that increased evaporation of the tear film, due to a compromised lipid layer, is one of the most common etiologies for the hyperosmolarity of the tear film and therefore highly responsible for the arising of keratoconjunctivitis sicca. Thus, a group of studies has focused on the investigation of the tear film lipid layer.[21,22] Goto and Tseng[22] have shown that the spreading time of the lipid layer after a blink is significantly prolonged in patients with DED compared with healthy subjects. These findings were extended by a second study on the lipid layer behavior, which investigated the dynamics of this outermost layer before and after a punctual occlusion.[23] This study reported that the lipid layer stabilization time was significantly reduced after this therapeutic procedure, indicating that the performance of the lipid layer is strongly influenced by the underlying aqueous tear fluid. Additionally, the lipid layer spreads more uniformly immediately after a blink in healthy eyes.

To further investigate this potentially useful parameter, we developed a novel device based on interferometry and compared the results with the standard diagnostic procedures for DED. Thus, the principal purpose of the current study was to find an objective, robust, and noninvasively assessable parameter for the evaluation of the tear film lipid layer that can be used for clinical routine. Because none of the currently common clinical dry eye tests objectively checks the functional status of the lipid layer, we believe that such a test would substantially enhance the diagnosis of patients with DED by providing a quantitative description of the specific performance of the tear film lipid layer.

Materials and Methods

Data were obtained from a prospective clinical study. Participants were chosen from the dry eye unit and from the staff of the Department of Ophthalmology, Medical University Graz. Subjects were excluded from the study if they showed symptoms of an active inflammation of the eye, any types of lid deformation, or if they had had ocular surgery within 6 months before the study took place. The experiments were approved by a local ethics committee and were in accordance with the 1964 Declaration of Helsinki. All subjects gave their informed consent to participate before their inclusion in the study. A total of 59 subjects ($n = 45$ female, $n = 14$ male) were included in the study. The subjects were divided into three subgroups, based on their subjectively reported severity as determined with the OSDI[24]: Subgroup 1 (mild, OSDI: 0–15, gender: 15/4 female/male, age: 55.2 ± 16.4 a), Subgroup 2 (moderate, OSDI: 16–30, 11/6 female/male, 52.9 ± 13.6 a), Subgroup 3 (severe, OSDI: 31–100, 19/4 female/male; 58.65 ± 13–a). The ?? distribution of the study population is shown in Figure 1.

Subjects were instructed not to use artificial tears or other local medications relevant to the tear film for at least 2 hours before the investigation. Contact lens wearers ($n = 5$) removed their contact lenses at least 8 hours before their examination. To characterize the subjects, tests were performed in the following sequence: OSDI, noninvasive lipid layer movement, osmolarity, TFBUT, lissamine green staining of the ocular surface, and Schirmer test (without anesthesia). All tests except the noninvasive evaluation of the lipid layer movement were performed on both eyes.

Lipid Layer Dynamics

To investigate the lipid layer dynamics, we developed a new diagnostic device (Fig. 2). This device illuminates the eye with a diffuse white light source, thereby making the lipid layer visible based on the physical principle of "white light interferometry," as previously described by Guillon.[25] Images were acquired via a USB microscope (PCE-MM200 Microscope, PCE Group, Meschede, Germany) and digitized as uncompressed audio-video interleaved (AVI) files at a spatial resolution of 640×480 pixels and at a temporal resolution of 20 frames per second, resulting in an interframe interval of 50 ms. The brightness was considered to be low enough, as no patients reported photophobic sensations during the investigation. The required certified medical device tests ensured that the illumination level was safe for the patients.

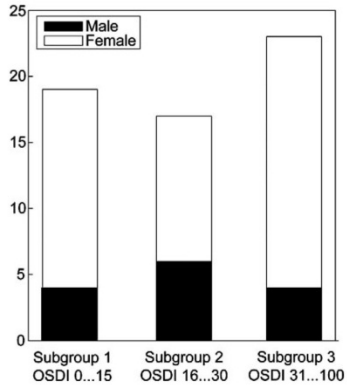

Figure 1. Distribution of the subjects.

The lipid layer stabilization time of five consecutive blinks was determined manually as the time between the beginning of the opening phase of the blink and the time when the movement of the lipid layer had stopped. The median value of five consecutive blinks was used for further analysis.

One representative image sequence of one subject is shown in Figure 3. Each subplot shows the interference image of the lipid layer. The first image is the onset of the opening phase of the blink; the last image represents the frame where no distinct movement of the lipid layer could be observed any more. The resulting, uncorrected stabilization time in this case was 2.1 seconds. Although the opening state of the eye has a direct influence on the tear film, we need some calibration parameter to correlate the distance between upper and lower eyelids, expressed in pixels, to the anatomical size of the eye. To achieve such a normalization, we chose to correct the lipid layer stabilization time by the ratio between the eyelid distance and the diameter of the iris. This compensates not only for differences in the size of the eye, but also for different distances between the camera and eyeball. Thus, the first step in our analysis was to fit a circle to the iris so as to extract the iris diameter. Subsequently, the distance of the eyelid margins was acquired through a parabola fit to each the upper and lower eyelid. The difference in vertical direction from the maximum value of the upper eyelid's parabola to the minimum of the lower eyelid's parabola was then taken as the eyelid distance in pixels (Fig. 4).

The measured lipid layer stabilization time was then corrected for the different eyelid opening states according to the following equation:

$$Tcorr[seconds] = Tmeasured[seconds] * \frac{IrisDiameter[pixels]}{LidDistance[pixels]}$$

Tear osmolarity was measured with the TearlabOsmolarity System (Ocusense, San Diego, CA). A small sample of tear fluid was taken from the lower tear meniscus of each patient using a pen. The bottom of the tip thereby came into contact with the thin line of moisture between the lower eyelid and the eye. Fluid was collected at the bottom tip of

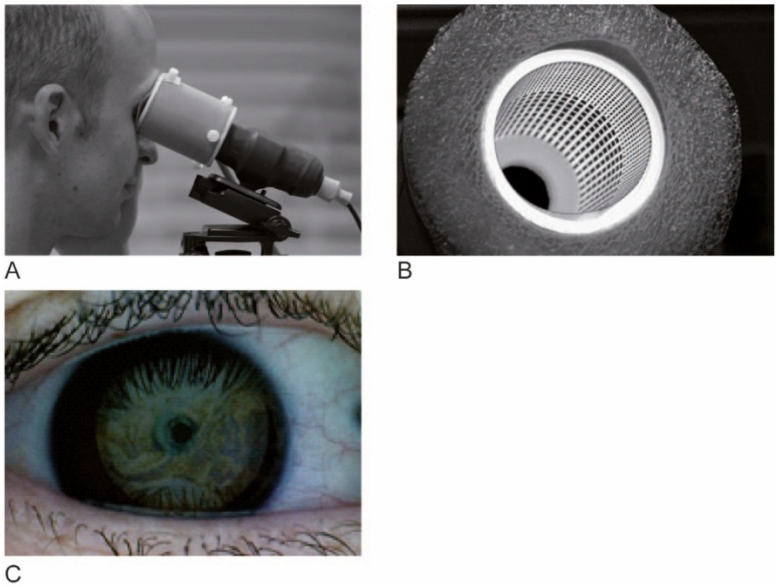

Figure 2. (**A**) The correct placement of the device during the investigation; (**B**) a close-up image of the opening of the device; (**C**) the resulting image.

the test card and the result was displayed after a few seconds. The measurement range is linear from 275 to 400 mOsms/L.[12,26]

TFBUT was measured by touching the inferior temporal bulbar conjunctiva with a fluorescein sodium strip, wetted with a preservative-free isotonic sodium chloride solution. Patients were instructed to blink. The precorneal tear film was then examined under blue-light illumination using a biomicroscope (Manufacturer, city, state/country) with a 10-fold magnification. The mean value of a total of three measurements was recorded.

Sterile strips impregnated with lissamine green (HUB Pharmaceuticals, LCC, Rancho Cucamonga, CA) were used to classify the exposed interpalpebral portions of the nasal and temporal conjunctiva and the cornea. The extent of staining was graded according to the van Bijsterveld score of 0 to 3 (0, negative; 1, scattered minute; 2, moderate spotty; and 3, blotchy) for each zone, with a maximum score of 9.[27]

A 5-minute conventional Schirmer test without anesthesia was performed on closed eyes by placing a commercially available 5 × 35-mm paper strip (Haag-Streit, Harlow Essex, UK) over the lid margin at the junction of the middle and lateral third into the tear film.

Statistical Analysis

The correlation of the corrected and uncorrected lipid layer stabilization time with the other clinical parameters was determined through the calculation of Spearman's rank correlation coefficient. A

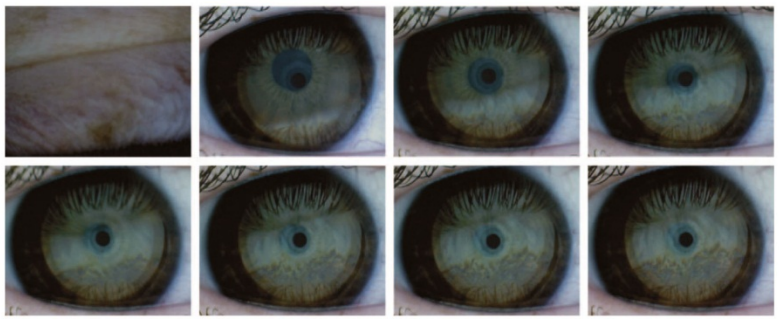

Figure 3. Representative image sequence from one subject. Starting from the opening phase of a blink sequence (*top left*), the lipid layer stabilization time was determined as the point where no visible movement could be observed any more (*bottom right*). The time difference between each of these images is 0.3 seconds, resulting in a lipid layer stabilization time of 2.1 seconds.

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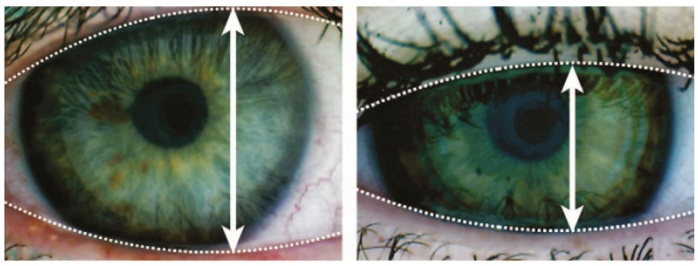

FIGURE 4. Large eyelid margin (*left*) compared with small eyelid margin (*right*) at the time of lipid layer stabilization.

linear regression analysis was conducted to quantify the relationship of the corrected lipid layer stabilization time and the other parameters.

Both the corrected and uncorrected lipid layer stabilization time, as well as the OSDI score, were checked for normality (Kolmogorov–Smirnov, $P > 0.05$; Q-Q-Plot). Because normality was not present, the nonparametric Wilcoxon signed rank test was used to check for significant differences between groups of different subjective severity. Significance level was set to $P < 0.05$ for significant correlations, and $P < 0.01$ for highly significant correlations. Statistical analysis was performed with the Statistics Toolbox of Matlab (MathWorks, Matlab, Natick, MA).

RESULTS

The linear relationship of the corrected lipid layer stabilization time and the subjectively reported severity for all subjects is shown in Figure 5. The correlation coefficient between OSDI and the corrected lipid layer stabilization time was $R^2 = 0.43$, $R^2 = 0.28$ without the correction. The correlation between the OSDI and the other parameters are as follows: R^2 TFBUT: 0.12, R^2 Schirmer test: 0.09, R^2 corneal lissamine staining: 0.08, R^2 osmolarity: 0.22. The mean value for the uncorrected lipid layer stabilization times within the groups of different severity were 1.11, 1.83, and 2.12 seconds, the corrected lipid layer stabilization time within the groups of different severity were 2.10, 2.68, and 3.62 seconds, respectively. The graphical demonstration of these parameters and the distribution of the values within the groups can be seen in Figure 6. The difference of the new parameter is highly significant between severe (OSDI: 31–100) and mild (OSDI: 0–15) graded subjectively reported symptoms, and significant between moderate (OSDI: 16–30) and severe subjectively graded severity. No significance could be observed between subjects with mild and moderate OSDI values.

The correlation coefficients of the commonly performed procedures and both the uncorrected and the corrected lipid layer stabilization times are shown in Table 1. The correction factor was greater than 1 in most of the cases, because only six subjects had a larger distance of the eyelid margins than their iris diameter was at the time of the observation.

To check the repeatability of this parameter within each subject, we also determined the variance over the five blinks in each subject (overall mean of the corrected lipid layer stabilization time: 2.80 seconds): the mean value for this intrasubject variance was 0.38 seconds (SD: 0.35 seconds).

Since the mean difference between Subgroup 3 (severe) and Subgroup 1 (mild) was 1.52 seconds, the mean variance of the corrected lipid layer stabilization time was 23% of this subgroup difference. In comparison with the intergroup differences, the variance was 66% of the mean difference between Subgroup 1 and Subgroup 2, and 40% of the mean difference between Subgroup 2 and Subgroup 3. The specificity and the sensitivity at different thresholds were computed by constructing a receiver-operator characteristic curve. Our criteria for DED cases were an OSDI score of 15 or greater and, in addition, one of the following diagnostic characteristics: a Schirmer value of 5 mm or lower, a TFBUT of 5 seconds or lower, or a lissamine green staining score of 4 or greater. A cutoff value of 2.6 seconds results in a specificity of 82% and a sensitivity of 70%.

DISCUSSION

We have developed a novel parameter to extend the diagnostic possibilities for the multifactorial disease keratoconjunctivitis sicca, which we have called the "Corrected Lipid Layer Stabilization Time" (CLST). Due to the high variability of each of the commonly performed procedures, the existing diagnostic parameters typically have to be combined so as to obtain a reliable grading of the severity of the disease. Our proposed objective, noninvasively assessed parameter could provide valuable clinical information on the lipid layer of the tear film, thereby facilitating the accurate classification of this disease into subtypes. The dynamics of the lipid layer seem to be well correlated with the subjectively reported symptoms of the DED, especially in comparison with other parameters. This could be explained by the noninvasiveness and objectiveness of the procedure, which results in a low short-term variability of this parameter.

The variability in the subjectively perceived, as well as in objectively, commonly assessed parameters in dry eye patients makes it hard to determine the best way to classify these patients according to the severity of the disease.[12] To investigate the correlation between the lipid layer dynamics and the perceived severity of DED, we used here a classification based on the OSDI alone. Comparing the mean differences of the CLST values between the different subgroups, the short-term variability can be considered low enough to provide a good repeatability for the classification of severe DED. The separation of mild and moderate forms of DED is less clear (short-term variability is 66% of the mean difference of CLST of Subgroups 1 and 2). As a possible cutoff value for our group of subjects, we chose a CLST of 2.6 seconds. The resulting specificity and sensitivity scores of 82% and 70%, respectively, indicate a good performance of this parameter for the diagnosis of DED, although we would expect to have better performance when combining the proposed CLST values with other common diagnostic procedures.

The significant correlation of the CLST with the TFBUT indicates the importance of a fast lipid spread for the overall stability of the pre-ocular tear film; a reduced dynamic of this layer is often related to an instable tear film. Our results

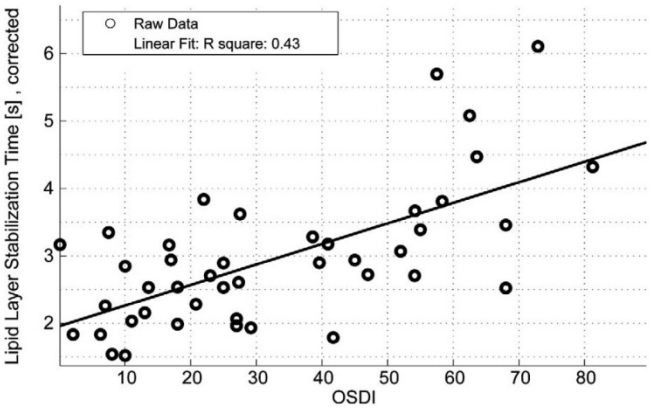

Figure 5. The linear regression model of the corrected lipid layer stabilization time and the subjectively reported severity.

confirm the data obtained by Goto and Tseng[23] and Yokoi et al.[28] In these studies, it was proposed that the spread of the lipid layer could be related to the amount of tear liquid in the aqueous compartments in the tear film. Our results strengthen this hypothesis by indicating a significant negative correlation with the Schirmer test. This tendency shows that in many cases a high Schirmer value results in a short stabilization time of the lipid layer. This is particularly interesting for choosing the most suitable therapeutic intervention. For example, a high Schirmer value, combined with a high CLST, would indicate a problem with the lipid layer, not with the aqueous layer. In that case, a treatment with lipid-containing tear substitutes could be the preferred solution over a treatment with lubricant eye drops.[29]

The background of the kinetics of the tear film lipid layer spread after a blink action has been reviewed by Bron et al.[3] and Butovich.[2] With the upward movement, the superior tarsal plate draws lipids from the region between the apposed lids. This leads to a rapidly upward propagating lipid layer over the underlying aqueous layer, which finally decays to a stabilization of the tear film lipid layer. It is supposed that initially the polar lipids spread rapidly over the aqueous layer, followed by a retarded movement of the nonpolar lipids.[28] Owens and Phillips[30] described the decay of the lipid spread velocity by a logarithmic function. They investigated the tear film lipid layer spread through the observation of particle movement in the tear film of healthy adult subjects. The lipid layer movement has been shown to stabilize after approximately 1 second, which is comparable to the uncorrected lipid layer stabilization time acquired by our device within Subgroup 1 (mild). Yokoi et al.[28] used a video interferometer (DR-1, Kowa, Tokyo, Japan)

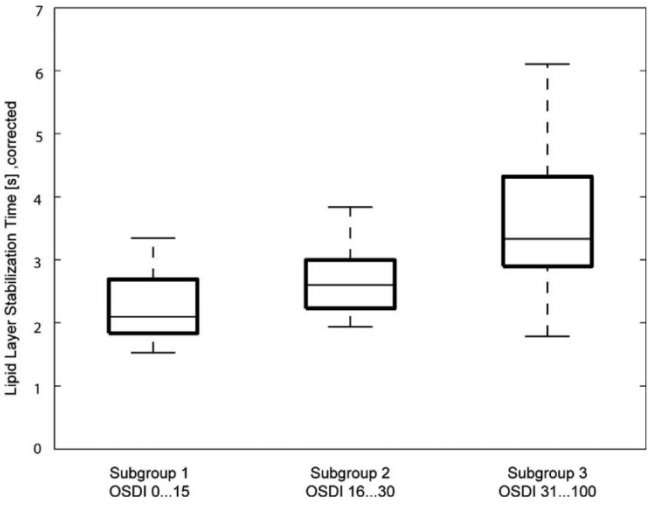

Figure 6. The location and scattering parameters of the different subgroups. Center-line boxes indicate the median and 25th and 75th percentiles, and the error bars indicate the measurement range.

TABLE 1. Correlations of the Uncorrected and the Corrected Lipid Layer Stabilization Time

	Lipid Stabilization Time Uncorrected		Lipid Stabilization Time Corrected	
	Rho	***P***	**Rho**	***P***
TFBUT	−0.303*	0.048	−0.485†	0.001
Schirmer I	−0.291	0.061	−0.431†	0.004
Lissamine green staining	0.223	0.165	0.379*	0.011
Osmolarity	0.074	0.659	0.004	0.861
OSDI	0.371*	0.017	0.498†	0.001

Spearman, $^{*}P \leq 0.05$; $^{\dagger}P \leq 0.01$.

for this purpose, and proposed a linear viscoelastic model as a theoretical attempt to describe the tear film lipid spread.

Even though we tested each of the 59 patients with each of the six tests, our study still has some limitations. For a complete investigation of our newly proposed parameter, a much larger and more heterogeneous patient population should be recruited, to check, for example, its variation with age. Although Bron et al.[3] have reported a good reproducibility of the tear film lipid layer dynamics, some patients have reported a clear change on their subjectively reported severity in the evening compared with the morning. Thus, an extension of our study to investigate the variation of this new parameter with the time of the day, as well as its reproducibility over a number of days would be interesting.

Because of the requirements from the ethics commission, we had to constrain our investigation with the new device in each patient to one eye only. The evaluation and comparison of both eyes could be of interest, as intrasubjective differences between the two eyes have been reported by some subjects. Nevertheless, we believe that this does not bias our results, as the behavior of the pre-ocular tear film acquired with binocularly performed standard procedures was highly correlated between the two eyes in each subject (R^2 TFBUT: 0.9, R^2 Schirmer: 0.8, R^2 osmolarity: 0.8). In six patients it was not possible to reliably acquire the lipid layer stabilization time, however. This was due to the low visibility of the tear film lipid layer pattern in these patients, who typically had prominent bright iris colors. We also observed that the visibility of the tear film lipid layer decreased clearly in patients with meibomian gland dysfuntion and primary Sjogrens syndrome. Although the stabilization time could be obtained reliably in most of these cases, the visibility was not high enough to extract the exact parameters of the kinetics of the tear film lipid layer (e.g., the initial velocity) from the images generated by our setup with the current configuration. For these purposes other devices seem to be more appropriate.[28]

Worth noting are also inherent limitations in the assessment of the subjective symptoms with the OSDI: the OSDI is among other criterions based on the visual performance. Because we included mostly elderly people into the study, a poor score in questions about visual impairments during daily activities (e.g., driving at night) may arise from other ophthalmic diseases, such as age-related macular degeneration or cataract. But because the OSDI has been extensively used by other studies and has been evaluated for its usage in dry eye diagnosis, we believe that this factor does not lead to a biased study selection.[24]

Although a slow lipid spread may be responsible for the hyperevaporation of the tear fluid, the correlation of the CLST and the osmolarity measurement was remarkably low. This could be explained by the fact that only one osmolarity measurement was performed for each eye in contrast to the proposed procedure of Khanal and Thomas.[31] They suggested taking the average of three consecutive measurements to reduce the variability of this test. The sensitivity and specificity of the osmolarity measurement are still under discussion.[32]

Although the sampling rate used in our study was much higher than in comparable previous studies,[22,23] it was still too low to reliably acquire the lid closure dynamics. Details of the lid closure dynamics could be of potential interest to investigate the dynamics of the lipid layer and the dynamics and completeness of eyelid closures in more detail.[33] To acquire a more detailed insight into the interaction between the lipid spread and the underlying aqueous layer, it would be necessary to couple our device with a method that measures the local thickness of the aqueous layer. This could be achieved through optical coherence tomography, for example. Due to the constraints of the different methods, the corresponding experiments would probably have to be conducted sequentially.

As an outlook, the possible connection of a slow lipid layer spread and transient changes in visual performance after a blink could be interesting, since the lipid layer is an important refracting layer for the incoming light. An inhomogeneous distribution could lead to chromatic aberration and image defects. For investigations concerning this question, a coupling of our procedure to methods proposed by Ridder et al.[15] or Benito et al.[14] would be necessary. They both describe different methodologies to assess the transient visual impairment associated with DED.

Our experiments and data analysis showed that for routine usage of the lipid layer dynamics in the diagnosis of DED patients, it would be desirable to have an autofocus system, and an automated data analysis of the acquired videos. Also, we want to make clear that whereas this new parameter provides valuable information about the lipid layer of the tear film, other tests are required for a complete assessment of a DED patient. Thus, the CLST should not be taken as the only parameter to assess the severity of the DED. Rather, we believe that this parameter should be seen as one step to acquire a comprehensive view on the complex construct of the tear film to grade the overall severity of the disease. By providing a direct measurement of the behavior of the lipid layer, the CLST can be valuable for the development of diagnostic and therapeutic patterns for different subtypes. In contrast to the subjective grading of the tear film integrity, our novel device provides an objective, noninvasively assessed parameter for the performance of the lipid layer.

We have also shown the importance of the lid opening on the behavior of the lipid layer. We think that this is important because there was a great variability within patients and it is supposed that a larger lid margin distance leads to a thinner tear film. It is possible that patients with a small quantity of tear fluid compensate for this by reducing the opening of the eye.

Our results underline the importance of the tear film lipid layer and potential of interferometric methods for the diagnosis of tear film performance, in particular if the performance of the lipid layer is corrected for the influence of different eyelid opening behaviors.

Acknowledgments

We thank ~~Dr~~ Falk Schrödl for his valuable advice and constructive criticism for the study design and the research. We also thank Michael Platz for his assistance in study design and data analysis.

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