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Doctoral thesis

Development of objective non-invasive techniques for the assessment of the ocular surface and tear film dynamics acting as biomarkers for early diagnosis of dry eye disease

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Tesis doctoral

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PROGRAMA DE DOCTORADO EN OPTOMETRÍA Y CIENCIAS DE LA VISIÓN

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Development of objective non-invasive techniques for the assessment of the ocular surface and tear film dynamics acting as biomarkers for early diagnosis of dry eye disease

Thesis presented by

Izabela Katarzyna Garaszczuk

to apply for the Degree of

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Research presented in the following dissertation was realized in accordance with the project entitled: *Biomarkers' trends for early diagnosis of dry eye disease,* as a part of the European Dry Eye Network (EDEN) initiative, which has received funding from the European Union's Horizon 2020 Research and Innovation Programme, under the Marie Skłodowska-Curie grant agreement number 642760.

This project is within the work package of EDEN called: *Objective non-invasive diagnostic tools*. The main objective of this work package is to create new metrics and technology to objectively and precisely assess and analyse the lacrimal functional unit. This will improve differentiation between healthy and dry eye disease subjects and diagnose different aetiologies affecting the latter. EDEN is a joint-PhD initiative; thus, a part of this research has been developed and conducted at the Department of Biomedical Engineering and Instrumentation of Wroclaw University of Science and Technology, in collaboration with the Biomedical Signal Processing Group, under the supervision of professor D. Robert Iskander.

DECLARATION

This dissertation is the result of my own work and includes nothing, which is the outcome of work done in collaboration, except where specifically indicated in the text. No portion of the work referred to in the following dissertation has been submitted in support of an application for another degree or qualification of this or any other University or other institution of Learning.

_____

Izabela Katarzyna Garaszczuk, MSc

Burjassot, 2018

Alejandro Cerviño Expósito, Assoc. Prof, and **Prof. Robert Montés Micó,** from the University of Valencia and **Prof. D. Robert Iskander** from Wroclaw University of Science and Technology, **CERTIFY** that the present report entitled: "*Development of objective non-invasive techniques for the assessment of the ocular surface and tear film dynamics acting as biomarkers for early diagnosis of dry eye disease*", summarizes the research work carried out under their supervision, by **Izabela K. Garaszczuk, MSc** and constitutes her thesis to apply for the double interdisciplinary degree of Doctor of Philosophy in Optometry and Vision Sciences at the University of Valencia and Biocybernetics and Biomedical Engineering at Wroclaw University of Science and Technology.

And to make it be on record, and complying with current legislation, they sign the present certificate in _____, on the _____ day of _____ of the year_____

Alejandro Cerviño-Expósito	Robert Montés-Micó	D. Robert Iskander

DEDICATION

"The good life is one inspired by love and guided by knowledge"

~ Bertrand Russell

My humble effort I dedicate to my parents for giving me strength and motivation to chase my dreams.

~~~~

I would like to express my cordial gratitude to my beloved fiancé for his understanding and making me smile in the moments of doubt and to my dear friend - Maryam Mousavi, for her positive energy and empathy, that uplift my spirit. I sincerely thank my supervisors: Alejandro Cerviño-Expósito and Robert Montés-Micó, for their guidance and confidence in me and to D. Robert Iskander for his support and being my constant source of inspiration. To my brother - Adam and to all of you, who had been there for me when I needed your support. To Clara, Edouard, Vincent, Laura, Duygu, Daniel, Mary, Anna, Mateusz, grandmother Maria, Dorota, Monika, Georgios, Eleni, Danilo, Przemek, Mariusz, Biomedical Signal Processing Group in Wroclaw and to Optometry Investigation Group in Valencia.
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LIST OF ABRREVIATIONS AND ACRONYMS

BR: Blink Rate

CCT: Central corneal thickness

DED: Dry eye disease

DEQ-5: 5-item Dry Eye Questionnaire

DEWS: Dry Eye Workshop report

EDEN: European Dry Eye Network

ESP: Eye Surface Profiler

FBUT: Fluorescein tear film break-up time

FCT(s): Fluorescein tear clearance test(s)

Hy: Hydrogel

K5M: Oculus Keratograph® 5M

LFU: Lacrimal functional unit

LLT: Lipid layer thickness

LWE: Lid wiper epitheliopathy

McMQ: McMonnies questionnaire

MGD: Meibomian gland dysfunction

NIKBUT: Non-invasive Keratograph 5M® Tear Film Break-up Time

F-NIKBUT: First non-invasive Keratograph 5M® tear film break-up time

M-NIKBUT: Mean non-invasive Keratograph 5M® tear film break-up time

OCT: Optical coherence tomography

OSDI: Ocular Surface Disease Index

SiHy: Silicone-hydrogel

TCR: Tear clearance rate

TCR_{TMA}: Tear clearance rate based on tear meniscus area

TCR_{TMD}: Tear clearance rate based on tear meniscus depth

TCR_{TMH}: Tear clearance rate based on tear meniscus height

TFWR: Tear fluorescein wash-out rate

TMA: Tear meniscus area

D-TMA: Dynamic meniscometry-based tear meniscus area

S-TMA: Tear meniscus area based on single, static OCT scan

TMD: Tear meniscus depth

D-TMD: Dynamic meniscometry-based tear meniscus depth

S-TMD: Tear meniscus depth based on single, static OCT scan

TMH: Tear meniscus height

D-TMH: Dynamic meniscometry-based tear meniscus height

S-TMH- Tear meniscus height based on single, static OCT B-scan

TMH_{K5M}: K5M-based tear meniscus height

TTR: Tear turnover rate

RESUMEN (ESPAÑOL)

Resumen (Español)

La siguiente disertación supone un trabajo de largo alcance con el propósito de encontrar un nuevo biomarcador de tipo macro que podría ser determinado de forma objetiva y no invasiva como indicador de ojo seco (DED), específicamente en su estado temprano. Para lograr este objetivo, se proponen nuevas métricas de cuantificación de la dinámica lagrimal y se explora el papel fisiopatológico de biomarcadores potenciales en el DED incipiente, mediante el seguimiento visita a visita de tendencias longitudinales de estas medidas durante un periodo de tiempo de un año, en sujetos con sintomatología incipiente.

La hipótesis tras el desarrollo de nuevos biomarcadores de tipo macro de DED es que la pérdida de *homeostasis de la película lagrimal*, que describe el mecanismo fisiopatológico esencial del DED, puede ser expresada no solo como una morfología alterada de la película lagrimal, sino también por una falta de equilibrio entre los procesos hidrodinámicos que ocurren en fluido lagrimal o meniscos lagrimales.

Estos fenómenos están en un estado de equilibrio regulado por la unidad funcional lagrimal. Una disrupción en este sutil equilibrio llevará en último término a DED. Basándose en la observación anteriormente mencionada, la tasa de eliminación lagrimal (TCR) fue seleccionada como un potencial macro-biomarcador de DED. La naturaleza multifactorial del DED puede ser expresada por la TCR, dado que considera todos los fenómenos hidrodinámicos que ocurren en el fluido lagrimal y ha mostrado funcionar bien en el diagnóstico diferencial de DED.

En este trabajo, varias medidas oculares y protocolos que podrían ser interpretados y utilizados como potenciales cuantificadores de TCR han sido desarrollados. Es más, algunas de las medidas oculares ya existentes han sido identificadas como

biomarcadores potenciales de DED. Estas medidas y cuantificadores, que pueden ser determinados de forma no invasiva y analizados objetivamente, son apropiados para completar la definición de DED y, lo que resulta de mayor importancia, pueden ser evaluados en un contexto clínico.

Esta tesis doctoral ha sido dividida en dos partes principales: *Parte experimental* y *Estudio de tendencia de biomarcadores*. El capítulo experimental (*Capítulo II*), en forma de tres experimentos separados, propone nuevas metodologías para la cuantificación dinámica de la lágrima. La segunda parte (*Capítulo III*) describe el estudio longitudinal de un año de duración, que fue diseñado para seguir las tendencias visita a visita de los biomarcadores anteriormente mencionados. Es de interés desarrollar metodologías más simples y, por extensión, más aplicables clínicamente para la cuantificación de la eliminación lagrimal.

Las técnicas descritas en el capítulo experimental pueden ser utilizadas para seguir, analizar y cuantificar diferentes aspectos de la dinámica del fluido lagrimal. Las medidas temporales de eliminación y renovación lagrimal fueron referidas en varios estudios como marcadores de la integridad de la unidad funcional lagrimal (LFU) e intercambio lagrimal sobre la superficie ocular. Dichas medidas tienen el potencial de convertirse en los nuevos macro-biomarcadores de DED. Considerando la probable aplicabilidad de las medidas de TCR en el apoyo al diagnóstico de DED, esta disertación propone tales nuevas soluciones en forma de tres experimentos separados. Describe dos metodologías alternativas para la determinación de TCR – una que emplea el instrumento recientemente desarrollado para la determinación de topografía corneo-escleral para seguir el decrecimiento de intensidad de fluorescencia lagrimal tras instilación de fluoresceína (***Experimento 1***) y el último,

que permite la estimación de TCR en fase temprana (***Experimento 3***) con el uso de un algoritmo de meniscometría dinámica basada en tomografía óptica de coherencia (OCT) propuesto en *el* ***Experimento 2***, en el que tres métodos diferentes para medir los parámetros de menisco son comparados. Los métodos para evaluar la dinámica lagrimal han demostrado ser procedimientos de corta duración, fáciles de llevar a cabo, y clínicamente aplicables.

La perfilometría con fluoresceína (***Experimento 1***) puede ser utilizada para seguir cambios sutiles, dinámicos que ocurren en la película lagrimal en toda la superficie corneo-escleral expuesta y no está limitada por la permeabilidad corneal a la fluoresceína. El método ha mostrado también ser repetible.

Un software personalizado para determinar meniscometría dinámica (***Experimento 2***) fue propuesto como un modo de mejorar la precisión de las medidas de morfología de menisco lagrimal con OCT, minimizando el efecto de la no-confluencia tras cada parpadeo en las estimaciones referidas. La OCT puede ser utilizada como un método rápido, cualitativo y cuantitativo para la determinación de parámetros del menisco lagrimal y tasa de eliminación lagrimal. Con este nuevo algoritmo, los parámetros de menisco lagrimal pueden ser calculados de forma más precisa tras cada parpadeo. También se ha observado que la meniscometría dinámica proporciona al clínico con información diferente que la meniscometría basada en un único corte tomográfico tipo-B estático.

Las medidas basadas en OCT de la tasa de eliminación temprana (***Experimento 3***) son no-invasivas, relativamente rápidas y más simples de llevar a cabo que las pruebas lagrimales tradicionalmente utilizadas. La OCT permite una visualización de los

meniscos lagrimales y de la eliminación lagrimal en mayor profundidad. Básicamente, el estudio longitudinal fue organizado para seguir las tendencias de los biomarcadores de acuerdo con el principal objetivo de la presente disertación, con el TCR incluido en el protocolo.

Para observar los cambios temporales, longitudinales, en la fisiología ocular (estudio de tendencia de biomarcadores), se adaptaron lentes de contacto actuales a los voluntarios, desechables diarias de silicona hidrogel (SiHy, Delefilcon A) o hidrogel (Hy, Omafilcon A). El protocolo fue diseñado considerando las limitaciones de tiempo y costes. El suministro gratuito de lentes favoreció los resultados de asistencia, siguiendo un calendario sistemático, que hizo el diseño del estudio más robusto y minimizó el número de abandonos. La parte clínica del estudio duró 12 meses. La hipótesis que orientó este estudio longitudinal fue que el porte de lentes de contacto tendría impacto de alguna forma en la fisiología ocular en el transcurso del periodo de 12 meses, de modo que las tendencias de los biomarcadores podrías ser observadas.

El protocolo del estudio longitudinal consistió en una visita de cualificación (basal), la visita de adaptación de lentes de contacto (día siguiente – visita del día 2), una visita de adaptación de lente de contacto a las dos semanas y visitas de seguimiento a los tres meses, seis meses y doce meses tras la adaptación, seguidas por la visita de evaluación post-estudio tras tres días (Control). La temperatura (°C) y humedad (%RH) del laboratorio fueron monitorizados.

Las medidas más adecuadas para completar la definición de DED y sus subclasificaciones fueron seleccionadas a partir de aquellas que podrían ser determinadas en un entorno clínico. Por ello, el protocolo de medidas incluyó cronológicamente: índice de

enfermedad de superficie ocular (OSDI) y Cuestionario de Ojo Seco de 5-items, altura de menisco lagrimal (TMH) medida con Keratograph 5M (K5M), medidas de osmolaridad lagrimal adquiridas a partir del menisco lagrimal inferior con el TearLab Osmolarity System®, tiempo de rotura lagrimal no invasiva con el Keratograph® (NIKBUT) y exploración de segmento anterior con lámpara de hendidura, siguiendo un protocolo estricto. Posteriormente, las medidas oculares propuestas en la parte experimental del proyecto fueron utilizadas como biomarcadores adicionales de DED. Ello incluyó meniscometría dinámica (estimación de altura, profundidad y área de menisco lagrimal) y determinación de TCR basado en la morfología dinámica del menisco lagrimal inferior adquirida con OCT. La cuantificación de TCR fue seguida por tinción palpebral con verde de lisamina y meibografía infrarroja obtenida con K5M. Las medidas fueron determinadas utilizando los métodos más objetivos, automatizados y clínicamente aplicables, utilizando radiación infrarroja y alternativas no invasivas a las medidas tradicionales, siempre que fue posible.

Cincuenta y cinco sujetos participaron durante toda la duración del estudio. La edad promedio del grupo fue de (media ± desviación estándar) 26 ± 4 años en un rango que comprendía desde los 20 a los 37 años. Basado en los criterios de adaptación de lentes de contacto llevado a cabo en el día 2, a 38 sujetos (25 mujeres/13 hombres) y a 17 sujetos (11 mujeres/6 hombres) se les adaptó lentes de contacto desechables diarias de hidrogel de silicona (SiHy) e hidrogel (Hy), respectivamente. Dado que no hubo diferencias estadísticamente significativas entre ojos derecho e izquierdo, adaptados con SiHy y Hy, hombres y mujeres, en ninguno de las métricas oculares medidas y las lentes tenían características de borde similares, el grupo de sujetos fue unificado en una única cohorte y los cambios temporales en tendencia de biomarcadores fueron analizados para el grupo

de estudio en conjunto. La limitación para este estudio fue la falta de control en las condiciones ambientales del estudio. Los cambios en las condiciones ambientales pueden ser debidos a cambios estacionales y no pudieron ser evitados en este estudio. Sin embargo, no se encontró correlación estadísticamente significativa entre la temperatura del laboratorio y humedad con ninguna de las medidas oculares evaluadas, excepto para la valoración de epiteliopatía palpebral. El análisis no paramétrico ANOVA mostró tendencias temporales estadísticamente significativas en OSDI y DEQ-5 en sujetos con sintomatología incipiente, en osmolaridad lagrimal, tiempos de rotura lagrimal objetiva no invasiva M-NIKBUT y F-NIKBUT, altura de menisco lagrimal evaluada mediante meniscometría dinámica y medidas de eliminación lagrimal y tinción con tinciones vitales, puntuaciones de epiteliopatía palpebral, espesor corneal y cuantificación de alteración de glándulas de Meibomio.

No se encontraron diferencias estadísticamente significativas entre las visitas basal y control en algunas de estas medidas, sugiriendo que los cambios temporales inducidos por las lentes de contacto y evaluadas con este método, podrían ser a corto plazo. Tampoco se encontraron diferencias estadísticamente significativas en los valores de enrojecimiento bulbar y limbal, lo que podría sugerir que los cambios observados en el estudio no son de naturaleza inflamatoria. En el transcurso del estudio, un decrecimiento gradual de la eliminación lagrimal fue probablemente debido a cambios en la morfología del saco conjuntival. El área de menisco lagrimal está significativa y negativamente correlacionada linealmente con la osmolaridad lagrimal. Adicionalmente, el método de meniscometría dinámica ha probado ser lo suficientemente sensible para revelar cambios potenciales en la forma de la superficie ocular.

En resumen, la presente tesis propone nuevas métricas de cuantificación de la dinámica lagrimal y explora el papel fisiopatológico de varios biomarcadores oculares de la homeostasis lagrimal. A través de las tendencias visita a visita de varios biomarcadores potenciales durante un periodo de un año, se muestra que la osmolaridad lagrimal puede ser utilizada para seguir cambios sutiles en la homeostasis de la película lagrimal. Los cambios en la osmolaridad lagrimal se correspondieron con el efecto beneficioso del porte de lentes de contacto sobre la película lagrimal tanto en portadores habituales de lentes de contacto sintomáticos, como en sujetos inicialmente clasificados como asintomáticos. Todos los cambios en la osmolaridad lagrimal durante el periodo de un año fueron estadísticamente significativos. Adicionalmente, la meniscometría basada en cambios dinámicos en la altura de menisco lagrimal parecía responder a cambios muy sutiles en los parámetros de menisco lagrimal durante el tiempo de desarrollo del estudio. Estos cambios fueron expresados adicionalmente como cambios en la eliminación lagrimal basada en OCT. En resumen, este estudio propone nuevos métodos de evaluación de la eliminación y reemplazo lagrimal y muestra como la osmolaridad lagrimal, eliminación lagrimal y meniscometría dinámica podrían ser utilizados como potenciales biomarcadores para apoyar el diagnostico de DED, y son lo suficientemente sensibles para seguir la progresión de cambios sutiles con el tiempo y la respuesta a terapia efectiva.

SUMMARY (ENGLISH)

The following dissertation is a far-reaching work with the purpose to find a new macro-type biomarker that could be objectively and non-invasively measured as an indicator of DED, specifically at its early stage. Attempting to achieve this goal, it proposes new metrics of tear dynamics quantification. By tracking visit-to-visit longitudinal trends of these measures over a period of one year, it explores their role in supporting DED diagnosis in subjects reported with incipient symptomatology.

The hypothesis behind developing the new macro-type biomarkers of DED is that the *loss of homeostasis of the tear film,* which describes the core pathophysiological mechanism of DED, may not only be expressed as disturbed tear film morphology, but also by a lack of equilibrium between hydro-dynamic processes occurring in the tear fluid or tear menisci. These phenomena are in a state of equilibrium regulated by the lacrimal functional unit (LFU). A disturbance of this subtle balance may ultimately lead to DED. Based on the abovementioned observation, the tear clearance rate (TCR) and tear turnover rate (TTR) were chosen as the potential macro-type biomarkers of DED. The multifactorial nature of the disease can be expressed by TCR or TTR, as they consider all the hydrodynamic phenomena occurring in the tear fluid and were shown to perform well in DED differential diagnosis.

Several ocular measures that could be interpreted as potential quantifiers of TCR or TTR, have been developed. Further, several existing ocular measures have been identified as potential DED biomarkers. These measures and quantifiers can be non-invasively assessed and objectively analysed and thus are appropriate to fulfil DED definition and, what is most important, can be assessed in a clinical setting. This includes tear osmolarity,

meniscometry, tear film stability assessment with non-invasive, objective technique and TCR estimation with optical coherence tomography (OCT).

This dissertation is divided into two major parts: ***Experimental part*** and **the *longitudinal study of biomarkers' trends*.** The experimental chapter (***Chapter II***), in a form of three separate experiments, proposes new methodologies for tear dynamic quantification. The second part (***Chapter III***) describes the one-year-long longitudinal study, which was arranged to follow the above-mentioned visit-to-visit biomarkers' trends.

It is of interest to develop simpler and, by extension, more clinically applicable methodologies for TCR quantification. Techniques described in ***Chapter II*** can be used to follow, analyse and quantify different aspects of tear fluid dynamics. Temporal measures of TCR and TTR were reported in several studies as markers of the integrity of the LFU and tear exchange on the ocular surface. These measures have the potential to become the new macro-type biomarkers of DED. Considering the probable applicability of TCR measurements in supporting DED diagnosis, this dissertation proposes such new solutions. It describes two alternative methodologies for TCR assessment - one that utilizes the newly-developed device for corneo-scleral topography to follow tear fluorescence intensity decay after fluorescein instillation (***Experiment 1***) and the latter, which allows estimation of an early-phase TCR (***Experiment 3***) with the use of spectral-domain OCT-based dynamic meniscometry algorithm proposed in the ***Experiment 2***.

To observe temporal, longitudinal changes in ocular physiology (***Chapter III),*** subjects were fitted with modern, daily disposable Silicon Hydrogel (SiHy, Delefilcon A) or Hydrogel (Hy, Omafilcon A) soft contact lenses. Free supply of lenses aided attendance

outcomes and ensuing a systematic schedule, which made the study design more robust and has minimized the number of drop-outs. The clinical part of the study lasted for 12 months. The hypothesis driving this longitudinal study was that contact lens wear will somehow impact ocular physiology over the period of 12 months, so the biomarkers' trends can be observed. The longitudinal study protocol consisted of:

- qualifying visit (Baseline visit);
- contact lens fitting visit (following day - 'Day 2' visit);
- contact lens fit control at two weeks after refitting;
- follow-up visits at three months, six months and 12 months post-refitting;
- post-study assessment after three days (Control visit).

Laboratory temperature (°C) and humidity (%RH) were monitored. The most appropriate ocular measures to fulfil the definition of DED and its sub-classifications were chosen from the ones that could be estimated in a clinical setting. Thus, the protocol of measurement included chronologically:

- Ocular Surface Disease Index (OSDI) evaluation and calculation;
- 5-Item Dry Eye Disease Questionnaire (DEQ-5);
- tear meniscus height (TMH) measurements with Oculus Keratograph 5M (K5M);
- tear osmolarity measurements acquired from the inferior tear meniscus with TearLab Osmolarity System;
- non-invasive tear film break-up times (NIKBUT) assessment with K5M;
- slit lamp anterior eye examination.

Subsequently, ocular measures proposed in the experimental part were used as additional DED biomarkers, including:

- dynamic meniscometry (tear meniscus height, depth and area estimation) assessed with OCT;
- TCR assessment based on dynamic tear meniscus morphology

and was followed by:

- ocular surface and lid wiper staining with lissamine green and fluorescein;
- infrared meibography recording with K5M.

Measurements were performed with objective, automated and clinically applicable methods, using infrared radiation and non-invasive alternatives to traditional measures, whenever possible.

The methods for assessing tear dynamics were proven to be not time-consuming, easy to perform and clinically applicable. Fluorescein profilometry (***Experiment 1***) can be used to follow subtle, dynamic changes occurring in the tear film on the whole exposed corneo-scleral surface and the measurements are not limited by corneal permeability to fluorescein. The method was also reported repeatable. A custom-written software allowing dynamic meniscometry (***Experiment 2***) was proposed to enhance the precision of tear meniscus morphology measurements with OCT, by minimizing the effect of tear meniscus nonconfluence after each blink on the reported estimations. OCT can be used as a rapid, qualitative and quantitative method of determining tear meniscus parameters and TCR. With this new algorithm, tear meniscus parameters can be calculated more precisely. It was also observed that the dynamic meniscometry method may provide clinicians with different information than the meniscometry performed based on static, single OCT B-scan.

OCT-based measurements of early-phase TCR (***Experiment 3***) are non-invasive, relatively rapid and simpler to perform than the traditionally used tear exchange tests. OCT allows more in-depth visualization of tear menisci and TCR observation.

Fifty-five subjects participated for the whole duration of the longitudinal study. The group mean age was (mean ± standard deviation) 26 ± 4 y/o and was ranging from 20 to 37 y/o. Based on the contact lens fitting procedure, 38 subjects (25 females and 3 males) were fitted with Silicone-Hydrogel (SiHy) and 17 subjects (11 females and 6 males) were fitted with Hydrogel (Hy) daily disposable soft contact lenses.

Since there were no statistically significant differences noted between right and left eye of each subjects and between SiHy and Hy-fitted group or the group of males and females in any of the assessed ocular measures, the group of subjects was unified into one cohort and temporal changes of biomarkers' trends were analysed for the whole study group. Non-parametric two-way ANOVA showed statistically significant temporal trends in OSDI and DEQ-5 in subjects with incipient symptomatology and temporal changes in tear osmolarity, non-invasive objective measures of tear film break-up time (M-NIKBUT and F-NIKBUT), tear meniscus height assessed with dynamic meniscometry, TCR and in staining with vital dyes, lid wiper epitheliopathy (LWE) scores, corneal thickness and quantification of Meibomian gland drop-out.

Statistically significant difference between Baseline and Control visit was not noted in some of these measures, suggesting that the temporal changes induced by contact lenses could be short-term. No statistically significant differences were noted in bulbar and limbal redness scores, which may suggest that changes observed in the study are not of inflammatory nature.

Over the time-course of the study, a gradual decrease of tear clearance and tear osmolarity was observed. Tear meniscus area significantly negatively linearly correlated with tear osmolarity. Additionally, dynamic meniscometry method was proven to be sensitive enough to reveal potential changes in ocular surface shape.

This dissertation proposes new metrics of tear dynamics quantification and explores the pathophysiological role of several ocular biomarkers of tear homeostasis. By tracking visit-to-visit trends of several potential biomarkers over the period of one year, it shows that tear osmolarity may be used to track slight changes in tear film physiology. Changes in tear osmolarity corresponded with the beneficial effect of contact lens wear on the tear film both in habitual, symptomatic contact lens wearers and in subjects initially reported as asymptomatic. All changes in tear osmolarity over a period of one year were statistically significant. Additionally, meniscometry based on dynamic changes of tear meniscus height seemed to respond to very subtle changes in tear meniscus parameters over the time-course of the study. These changes were additionally expressed as changes in OCT-based TCR.

Summarizing, this dissertation proposes new methods of tear clearance and turnover assessment and shows that tear osmolarity, TCR and dynamic meniscometry measures could be used as potential biomarkers for supporting DED diagnosis, that are sensitive enough to follow the progression of subtle ocular changes in time and response to effective therapy.

STRESZCZENIE (POLSKI)

Niniejsza praca doktorska została wykonana zgodnie z założeniami projektu EDEN (European Dry Eye Network), finansowanego z programu Horyzont2020, w grancie Marii Skłodowskiej-Curie o numerze 6462760. Praca opisuje szeroko zakrojone badania mające na celu zidentyfikowanie lub wynalezienie nowych makro-biomarkerów, które zmierzone w sposób obiektywny i nieinwazyjny posłużą jako wskaźniki zespołu suchego oka (ZSO), w szczególności, w jego początkowej fazie.

W tym celu, niniejsza praca proponuje nowe miary dynamiki filmu łzowego w serii eksperymentów oraz dogłębnie śledzi jaką rolę odgrywają te nowe i inne, ustandaryzowane, miary w patofizjologii ZSO, poprzez śledzenie trendów tych markerów przez okres jednego roku u pacjentów z początkowymi lub umiarkowanymi objawami ZSO.

Główną hipotezą kierującą niniejszymi badaniami, jest stwierdzenie, że *spadek homeostazy filmu łzowego*, który to jest w literaturze opisany jako kluczowy patologiczny mechanizm u podstaw ZSO, może być wyrażony nie tylko jako zaburzenie proporcji poszczególnych składników, czy też objętości poszczególnych komponentów filmu łzowego, lecz także jako brak równowagi pomiędzy hydrodynamicznymi procesami zachodzącymi w filmie łzowym lub meniskach łzowych.

Wspomniane hydrodynamiczne procesy w zdrowym oku zachodzą w równowadze odpowiednej dla zachowania homeostazy filmu łzowego i adekwatnie do warunków środowiskowych, zdrowotnych i psychofizycznych, zaś regulowane są przez funkcjonalną jednostkę łzową (and. lacrimal functional unit, LFU). Zaburzenie któregokolwiek z tych procesów może trwale upośledzić tę równowagę i zapoczątkować rozwój ZSO.

Na podstawie powyższego założenia, współczynnik wymiany filmu łzowego (ang. tear clearance rate, TCR lub tear turnover rate, TTR) został wybrany jako potencjalny makro-biomarker ZSO. TCR i TTR tak jak i ZSO cechuje wieloczynnikowa natura. Wskaźniki te są proporcjonalne do wszystkich hydrodynamicznych procesów zachodzących w filmie łzowym i meniskach oraz wykazują potencjalne zastosowanie w diagnostyce różnicowej ZSO.

W niniejszej pracy badawczej zostało opracowanych kilka miar i protokołów pomiarowych, które mogą zostać zinterpretowane i użyte jako kwantyfikatory wymiany łez. Co więcej, kilka istniejących już miar zostało zidentyfikowanych jako potencjalne biomarkery ZSO. Te kwantyfikatory mogą być nieinwazyjnie zmierzone, obiektywnie przeanalizowane i mogą być stosowane w warunkach klinicznych. Tymi markerami są, w szczególności, osmolarność łez, parametry geometryczne menisku łzowego mierzone dynamicznie, stabilność filmu łzowego określona obiektywnie oraz TCR.

Niniejsza praca została podzielona na dwie główne części: ***Eksperymentalną*** i ***Studium trendów biomarkerów.*** W części eksperymentalnej (***Rozdział II***) zaproponowano nowe metody ilościowej analizy dynamiki filmu łzowego. Część druga (***Rozdział III***), opisuje proces przygotowania, przebieg oraz wyniki rocznej obserwacji trendów biomarkerów u osób noszących soczewki kontaktowe.

Ważnym jest, by opracować prostsze, przez co także, łatwiejsze w zastosowaniu klinicznym, metody ilościowej oceny dynamiki filmu łzowego, takie jak czynnik TCR. Techniki opisane w rozdziale eksperymentalnym mogą zostać użyte do śledzenia, analizy i ilościowego opisu różnego rodzaju aspektów dynamiki łez. TCR i TTR w literaturze zostały opisane jako miary integralności LFU oraz wymiany łez na powierzchni oka.

Te parametry mogą stać się potencjalnymi makro-biomarkerami ZSO. Kładąc nacisk na kliniczną przydatność metod opisanych powyżej, niniejsza praca proponuje nowe, alternatywne metody pomiaru dynamiki łez w formie trzech niezależnych eksperymentów. Opisuje ona dwie alternatywy dla tradycyjnych skomplikowanych i laboratoryjnych pomiarów TCR i TTR. Pierwsza z proponowanych metod polega na śledzeniu spadku intensywności fluorescencji fluoresceiny w filmie łzowym przy pomocy profilometrii rogówkowo-skleralnej (***Eksperyment 1***), druga zaś umożliwia estymację wczesnofazowej wymiany łez przy użyciu spektralnej optycznej koherentnej tomografii (ang. Optical coherence tomography, OCT) (***Eksperyment 3***), w oparciu o algorytm meniskometrii dynamicznej zaproponowany w ***Eksperymencie 2.*** ***Eksperyment 2*** porównuje trzy różne metody pomiaru geometrycznych parametrów menisku łzowego: metodę standardową *en face*, metodę opartą na statycznym, pojedynczym B-skanie OCT oraz nową metodę opartą na serii takich B-skanów (meniskometria dynamiczna).

Aby zaobserwować widoczne zmiany na powierzchni oka i w filmie łzowym w czasie, uczestnikom ***badań trendów biomarkerów*** zostały dobrane jednodniowe soczewki kontaktowe wykonane z materiału silikonowo-hydrożelowego (Delefilcon A) lub hydrożelowego (Omafilcon A), które uczestnicy nosili przez okres 12 miesięcy. Protokół badań podłużnych został przygotowany z uwzględnieniem ram czasowych i finansowych projektu EDEN. Darmowy zapas soczewek kontaktowych, który został przydzielony każdemu z uczestników, wpłynął na niezwykle mały współczynnik ich rezygnacji z udziału w badaniach, zaś stała kontrola przez specjalistę pozytywnie przyczyniła się do przestrzegania przez uczestników zaleceń względem pielęgnacji soczewek oraz czasu i częstotliwości ich noszenia. W związku z powyższymi

czynnikami, u większości uczestników zaobserwowaną pozytywne zmiany w fizjologii powierzchni oka oraz filmu łzowego. Główną hipotezą leżącą u podstaw powyższej metodologii, było założenie, że noszenie soczewek kontaktowych zaburzy w choćby minimalnym stopniu fizjologię powierzchni oka i filmu łzowego na przestrzeni jednego roku, dzięki czemu możliwe będzie zaobserwowanie trendów zaproponowanych biomarkerów.

Na protokół rocznych badań składało się 7 wizyt: wizyta bazowa wykonana na oczach bez soczewek, wizyta z dopasowaniem soczewek następnego dnia, wizyta kontrolna po dwóch tygodniach noszenia soczewek oraz właściwe wizyty pomiarowe po trzech, sześciu i 12 miesiącach noszenia soczewek kontaktowych, po których wykonano kolejne pomiary, po 3-dniowej przerwie w noszeniu soczewek. Temperatura i wilgotność powietrza w laboratorium pomiarowym była notowana osobno dla każdego uczestnika. Na pomiary składał się zestaw metod, które pozwalają na ilościowe i obiektywne określenie wszystkich procesów fizjologicznych wymienionych jako objawy ZSO, a które można zmierzyć w klinice i przy pomocy ogólnodostępnych urządzeń. Protokół zawierał zatem pomiary takie jak: kwestionariusz oceny chorób powierzchni oka (ang. Ocular Surface Disease Index, OSDI), skrócony kwestionariusz oceny zespołu suchego oka (5-item Dry Eye Questionnaire, DEQ-5), pomiar menisku łzowego w obserwacji *en face* przy pomocy Keratografu Oculus 5M® (K5M), pomiar osmolarności łez przy pomocy osmometru TearLab Osmolarity System®, nieinwazyjny pomiar czasu przerwania filmu łzowego przy pomocy K5M i ocena przedniego odcinka oka pod biomikroskopem z lampą szczelinową oraz pomiar grubości rogówki przy pomocy OCT. Kolejno wykonane zostały pomiary niestandardowe, zaproponowane w części eksperymentalnej niniejszej pracy, takie jak dynamiczna meniskometria z użyciem OCT

i estymacja TCR w oparciu o dynamiczną meniskometrię, by następnie ocenić uszkodzenie powierzchni oka na bazie pomiarów barwienia spojówki i rogówki zielenią lizaminową i fluoresceiną, oraz na bazie barwienia wycieraczki powiekowej i meibografii wykonanej w podczerwieni przy pomocy K5M.

Nacisk kładziono na to, aby, gdy to tylko możliwe, powyższe pomiary wykonać metodami gwarantującymi maksymalne zautomatyzowanie, powtarzalność, minimalną inwazyjność i obiektywność oceny oraz łatwość zastosowania w warunkach kliniczny.

Roczne pomiary trendów biomarkerów ukończyło 55 uczestników. Grupa składała się z młodych, zdrowych osób, których średni wiek to (średnia ± odchylenie standardowe) 26 ± 4 lata, w przedziale od 20 do 37 lat. Na podstawie procedury dopasowania soczewek, 38 osobom (w tym 25 kobietom i 13 mężczyznom) dopasowano jednodniowe, miękkie soczewki silikonowo-hydrożelowe, a pozostałym 17 osobom (w tym 11 kobietom i 6 mężczyznom) soczewki hydrożelowe.

Ponieważ nie było statystycznie znaczącej różnicy w żadnym z mierzonych parametrów pomiędzy grupami noszącymi różne soczewki lub też grupami rożnej płci, grupę badawczą ujednolicono i rozpatrywano całościowo w funkcji czasu. Nieparametryczna dwuczynnikowa ANOVA wykazała statystycznie znaczące trendy czasowe w OSDI, DEQ-5, osmolarności łez, nieinwazyjnych czasach przerwania filmu łzowego, dynamicznie mierzonej wysokości menisku łzowego, TCR oraz barwieniu powierzchni oka i tarczki powiekowej, a także w grubości centralnej rogówki i w meibografii. Brak zaobserwowanych statystycznie znaczących różnic pomiędzy pierwszą i ostatnią wizytą (które to zostały wykonane po przerwie w noszeniu soczewek) wskazuje na to, iż część zaobserwowanych zmian ma charakter przejściowy. Brak różnicy w

stopniu przekrwienia rąbka rogówki i spojówki gałkowej może sugerować, iż obserwowane zmiany nie mają charakteru zapalnego.

W trakcie trwania badań widoczny był stopniowy spadek TCR. Pole powierzchni przekroju menisku łzowego było negatywnie skorelowane z osmolarnością. Dodatkowo, dynamiczna meniskometria okazała się być metodą na tyle dokładną, by wykazać potencjalne zmiany w kształcie powierzchni oka i wpływ tych zmian na kształt menisku łzowego.

Metody pomiaru wymiany łez proponowane w niniejszej pracy są łatwe w wykonaniu, nieczasochłonne i obiektywne, przez co potencjalne mogą być stosowalne w środowisku klinicznym. Profilometr fluoresceinowy może zostać użyty do śledzenia subtelnych, dynamicznych zmian w filmie łzowym na całej powierzchni oka w granicy szpary powiekowej. Metoda ta nie jest ograniczona przez przepuszczalność rogówki dla fluoresceiny i cechuje ją relatywnie duża powtarzalność.

Algorytm postępowania i program komputerowy napisany w celu dynamicznego pomiaru menisku łzowego przyczyniły się do zwiększenia precyzji pomiaru parametrów geometrycznych menisku i dokładniejszej ich estymacji, odpornej na dynamiczne zmiany tych parametrów po każdym mrugnięciu. OCT może zostać użyte jako szybka i dokładna, jakościowa oraz ilościowa metoda oceny menisku łzowego oraz TCR. Dzięki zastosowanemu algorytmowi, parametry menisku obliczane są dokładniej, szybciej i automatycznie. W niniejszej pracy zaobserwowano także, że dynamiczna meniskometria dostarcza nowych informacji na temat subtelnych zmian zachodzących w menisku łzowym, które są poza zasięgiem standardowej metody pomiaru statycznego lub pomiaru *en face*. Ocena TCR przy pomocy OCT jest nieinwazyjna, relatywnie szybka i

dużo łatwiejsza w wykonaniu niż tradycyjnie metody używane do oceny wymiany i dynamiki filmu łzowego. OCT umożliwia bardziej wnikliwą wizualizację menisku oraz wymiany łez.

Niniejsza praca proponuje nowe miary homeostazy i dynamiki filmu łzowego oraz za ich pomocą eksploruje patofizjologiczny mechanizm ZSO. Śledząc trendy kilku potencjalnych biomarkerów na przestrzeni jednego roku wykazuje, że osmolarność łez może być używana jako wskaźnik subtelnych zmian w fizjologii filmu łzowego. Zmiany osmolarności łez korelują z wyindukowanym u pacjentów pozytywnym efektem zmiany soczewek kontaktowych na jednodniowe i bardziej nowoczesne. Pozytywne zmiany zostały zaobserwowane zarówno u osób symptomatycznych, jak i u tych bez objawów ZSO. Wszystkie zmiany w osmolarności na przestrzeni roku były statystycznie znaczące. Dodatkowo, meniskometria oparta na zaproponowanym algorytmie na bazie dynamicznych pomiarów zdaje się być dobrym wyznacznikiem subtelnych zmian parametrów menisku łzowego w czasie. Obserwowalne zmiany wyrażały się także jako spowolniony czas wypłukiwania łez, czyli obniżony TCR.

Podsumowując, niniejsza praca doktorska proponuje nowe metody pomiaru filmu łzowego oraz wykazuje, że osmolarność, TCR oraz dynamiczna meniskometria mogą być użyte jako potencjalne biomarkery wspierające diagnozę ZSO. Markery te są wrażliwe na subtelne zmiany fizjologii powierzchni oka u młodych, zdrowych osób, zatem z pewnością pozwolą na analizę owych zmian u osób z bardziej zaawansowaną manifestacją ZSO.

CONTENT

INTRODUCTION

Introduction

Dry eye disease (DED) is increasingly recognized as a worldwide public health concern[1]. Considering its growing incidence and morbidity, it is a matter of high importance to improve its timely diagnosis, to provide better treatment and prevention. By finding ocular biomarkers able to predict and assess DED progression, this diagnosis could be improved.

Biomarker can be defined as: *a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process or pharmacologic response to a therapeutic intervention*[2] or as *any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or a disease*[3].

This chapter provides a theoretical background on ocular measures used in DED diagnosis and management, limited to those markers that presumably have the highest potential to be used for supporting early DED diagnosis in clinical setting. Several ocular measures can be interpreted as such indications of DED, as they can be non-invasively assessed, objectively analysed and are appropriate to fulfil the DED definition. However, despite the interest to improve timely diagnosis of DED and several attempts, no one has yet found a singular objectively-measured macro-type marker that has the predictive value for DED diagnosis. This task has proven to be highly challenging, as the ocular measures used in DED assessment are generally characterized by lack of apparent correlation with each other and with subject-reported symptoms[4-6]. A battery of tests is usually performed to provide a reliable diagnosis[7]. Particularly at early stages of DED this diagnosis can be impeded by poor diagnostic test repeatability[8], significant portion of false-positive or false-negative rates[9], broad range of

variability, sensitivity and specificity[10] and by the dependence of these measures on environmental conditions[11] among many other factors.

Moreover, the commonly applied three-layered model of the tear film, proposed by Wolff[12] is a considerable simplification of tear film's true morphology[13,14]. Continual return to this model by researchers has limited the novel perspectives on understanding the complexity, dynamics, structure and function of the tear film, thus in the last decade only few researchers have aimed to identify the pathophysiological changes that occur in the tear film and ocular surface to cause DED[14].

Yet additional challenge lies in the multifactorial nature of the disease. Recently, the Tear Film and Ocular Surface Society Dry Eye Workshop II report (DEWS II, 2017), has defined DED as *a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological role*[15].

Regardless of the abovementioned difficulties, this dissertation attempts to identify biomarkers that can support DED diagnosis. The hypothesis driving the development of a new macro-type biomarker of DED is that the abovementioned *loss of homeostasis of the tear film*, describing the core pathophysiological mechanism of DED, may not only be expressed by disrupted tear film morphology, but also by a lack of equilibrium between hydrodynamic processes occurring in the tear fluid or tear menisci.

In a healthy eye, these hydrodynamic phenomena are in a state of equilibrium regulated by the lacrimal functional unit (LFU) composed of the ocular surface tissues, tear

secreting apparatus and their neural connections. Disruption of any of these hydrodynamic processes (e.g., increased evaporation or decreased tear secretion) will disturb this subtle balance and can lead to DED.

Based on the abovementioned observations, the tear clearance rate (TCR) was chosen as a potential macro-type biomarker of DED. It is multifactorial in nature; it considers all the hydrodynamic phenomena occurring in the tear fluid and was shown to perform well in DED diagnosis and classification[16].

A sufficient theoretical background on tear clearance and tear turnover rates is provided at the end of ***Chapter I***. Primarily, a point-by-point review of the ocular measures most promising as DED biomarkers is given. The order in which these measures are presented corresponds with their order of appearance in the recent definition of DED.

To summarize, this project is a far-reaching work attempting to find a new macro-type biomarker that could be objectively and non-invasively measured as an indicator of DED progression, specifically at its early stage. Trying to achieve this goal, it proposes new metrics of tear dynamics and explores the pathophysiological role of tear dynamics temporal characteristics in early DED aetiology and diagnosis. Ultimately, it explores the propensity to develop DED and tries to anticipate its evolution by tracking visit-to-visit trends of several proposed ocular biomarkers in a population with incipient symptomatology.

The initial hypotheses of this dissertation were that:

- DED is caused by a loss of homeostasis between the dynamic processes occurring in the tear film and menisci. By developing methods for objective, non-invasive

assessment of tear fluid dynamics, it will be possible to create new macro-type biomarker(s) for supporting early diagnosis of DED;

- By analysing trends of those several known or newly-developed ocular measures, it would be possible to predict the incidence of DED;

Based on these hypotheses, the study aims to address the following:

- To understand the role of some ocular biomarkers, particularly tear turnover rate, tear meniscus morphology and tear osmolarity in DED aetiology;
- to develop a non-invasive mode for objective tear film dynamics assessment and to propose a new marker of LFU's integrity, while showing whether these new measures:
    - can provide clinically valuable information;
    - can aid predicting DED;
    - correlate with other signs or symptoms of DED.

In accordance with the aim of this dissertation, it is divided into two major parts: ***Experimental part*** (***Chapter II*****),** where new solutions are tested and new methodologies for biomarkers assessment were proposed in a form of three, separate experiments, and the second part - ***the longitudinal study of biomarkers' trends (Chapter III*****)*** - describing the longitudinal protocol performed to follow the visit-to-visit biomarkers' trends and their role in supporting DED diagnosis.

The scope of the thesis is as follows: ***Chapter I*** serves as a theoretical background on the ocular measures used for DED diagnosis that could potentially be used as biomarkers. Techniques described in the experimental chapter (***Chapter II***, three separate

experiments) provide a mode for tracking, analysing and quantifying different aspects of tear dynamics. Further, ***Chapter III*** provides a comprehensive description of the study protocol, methodology and the results obtained over the time-course of ***the longitudinal study of biomarkers' trends*** in contact lens wearers. Ocular measures proposed in ***Chapter II*** were used as additional DED biomarkers in the longitudinal study. Finally, ***Chapter IV*** contains a detailed, comprehensive discussion of all the experiments performed and the biomarkers' trends reported in the longitudinal study.

CHAPTER I. THEORETICAL BACKGROUND

1.1. Tear film instability

The t*ear film instability* is defined as a major factor in DED aetiology[15]. Tear film break-up time (TBUT) - a temporal, indirect measure of tear film stability is the most commonly employed method for tear instability and tear film evaluation used in clinical practice[17]. TBUT is defined as the time interval between the last complete blink and the appearance of the first break in the tear film[18]. Fluorescein can be instilled to enhance visibility of break-ups, however, the dye may reduce tear film stability leading to decrease of measurements' accuracy[19,20]. For this reason, this method of assessment was not recommended as the first-choice method in DEWS II report.

Following the standardized methodology of tear film break-up time estimation is crucial. Generally, subject is asked to blink naturally three times and then to cease blinking until instructed. The reference value for DED diagnosis when fluorescein is used (FBUT) ranges from a cut-off time of less than 10 seconds[21,22] to less than five seconds when smaller, more controlled volumes of dye are instilled[20]. Mild and moderate DED subjects have a broad range of FBUT values and the diagnostic value of FBUT is less certain for these DED sufferers[8]. Despite the drawbacks of using fluorescein to assess tear film stability, inherent large variability of break-up times among subjects[23,24] and its dependence on environmental conditions, FBUT still remains one of the most commonly used diagnostic tests for DED in clinical practice[25]. Many different approaches have been proposed to improve repeatability of TBUT estimation, including taking multiple readings and averaging or selecting a subset of values[26,27], minimizing the amount of instilled fluorescein volume[28,29] or fully eliminating the use of fluorescein. The most common non-invasive approach is to project different patterns onto the tear film

surface and follow their distortion in time after the last natural blink[30-33]. These disturbances are related to changes in tear film surface quality[34,35], pre-lens tear film quality[36,37], tear film stability[38] and tear film break-ups[39]. Based on DEWS II report, the non-invasive technique (NIBUT) for tear stability assessment is considered preferable to FBUT[20,40]. Whenever possible, automated measurements are recommended[25] and the NIBUT cut-off value of ≤ 10s has been reported to be indicative of DED. NIBUT measurements have become more popular in both clinical practice and research, so that automated assessment of tear film stability is now possible. For example, the Keratograph 5M (K5M; Oculus Optikgerate Gmbh, Wetzlar, Germany), will be used in this project to provide objective and automatized method of tear stability assessment. It has three main advantages in assessing tear film break-up: the method does not require fluorescein instillation, it is almost fully automatic and utilizes infrared radiation, which minimizes the effect of reflex tearing and light scattering. K5M detects and maps locations of tear breakup over time[41,42] and can automatically detect and localize breaks and disturbances in the Placido disk pattern projected on the tear film surface. This method of assessment is objective, non-invasive and clinically applicable.

The link between hyperosmolarity and tear instability was reported, suggesting that transient increases in tear osmolarity may be observed under conditions of tear instability[43]. Increases in evaporation rates, resulting from prolonged interblink periods or as a result of environmental factors, can drive tear film break-up, and predict the increases in tear osmolarity at the centre of areas of rupture of the tear film[44].

1.2. Tear hyperosmolarity

Tear osmolarity has been described as a single clinical measure giving insight into the balance between tear production, evaporation, drainage and absorption[45]. Several studies support its use as a metric to diagnose and classify DED[14,25,46]. Tear osmolarity was reported as the least variable of all the common signs of DED across a clinically relevant timeframe and was the one to reduce its variation upon application of effective therapy[8,10].

Tear osmolarity refers to the amount of osmotically active particles in tears and is defined as the number of osmoles per litre of solution (mOsm/L)[47]. It is determined mostly by the electrolytes of the aqueous component of tears and less significantly by proteins and sugars[48]. Increased tear osmolarity (hyperosmolarity) is one of the core pathophysiological mechanisms of DED[9,15,49-51], that can cause damage of the ocular surface or initiate a chain of inflammatory responses leading to ocular surface damage[49,52], thus it was included in DED definition. Tear hyperosmolarity leads to increase of interferon gamma in the tear film, in the absence of corresponding increases of other Th1, Th2 and Th17 cytokines, which can induce epithelial cell apoptosis[53]. Its clinical evaluation was facilitated after introducing a chip-based osmometer (TearLab® Osmolarity System, TearLab Corp, San Diego, CA, USA)[54], which allows collection of a relatively small sample of 50nL from the inferior tear meniscus and automatic determination of tear osmolarity, based on the sample's electrical impedance. Before the introduction of an impedance-based osmometry, the method required relatively large volume of tears to be collected from the inferior tear meniscus and specialized laboratory osmometer to perform the analysis. Because of their invasiveness,

the difficulty to properly handle tear samples, the tear osmolarity measurements were rarely performed in a clinical environment. Its popularity as a standard clinical test for DED has grown significantly over the last decade.

The presumed difference in osmolarity between the tear film and tear menisci is fairly small, but it is predicted to increase for DED subjects, particularly when the evaporation rate increases together with reduced tear meniscus[55]. Tear osmolarity seems not to be related to aging[46,56-58], race[57,59,60] or hormonal fluctuation in women with a regular menstrual cycle[61,62]. However, data on the effect of gender on tear osmolarity[46,57,63,64] and its diurnal variations[56,59,65-67] remain equivocal. The average tear osmolarity among healthy adults was estimated to be around 302 ± 9.7 mOsm/L[25,46,61,63,65,66,68-72]. Jacobi et al.[71] using TearLab® osmometer reported values of around 301 mOsm/L, ranging from 298 to 304 mOsm/L. Tear osmolarity generally increases in DED and correlates with its severity score[9,14], from normal (302.2 ± 8.3 mOsm/L), through mild-to-moderate (315.0 ± 11.4 mOsm/L) to severe cases (336.4 ± 22.3 mOsm/L). Tear osmolarity of 308 mOsm/L is the most sensitive threshold to distinguish normal from mild/moderate forms of DED and 315 mOsm/L is the most specific cut-off value[9,46,71].

Additionally, the increasing inter-eye difference in osmolarity is considered an indication for the loss of tear film homeostasis in DED[10,46]. DED subjects report higher inter-eye variations of tear osmolarity compared to control groups[46,49,71,73]. In normal subjects the inter-eye variation of tear osmolarity is estimated to be around 6.9 ± 5.9 mOsm/L. An inter-eye difference in tear osmolarity was reported to decrease in response to successful DED treatment[8]. Inter-eye and intra-eye differences in tear osmolarity are the

additional factors aiding DED diagnosis as markers of the tear film's ability to maintain its homeostasis.

All these factors and characteristics prove great potential of tear osmolarity as a biomarker of DED. It seems to be utterly justified to include this marker in the study protocol and test its trends in subjects with incipient DED symptomatology and verify whether it can support diagnosis. However, some studies suggest high variability of the readings coming from mild/moderate DED or healthy subjects[74]. A relatively large overlap of values coming from such subjects is observed, which may ultimately lead to redefinition of the DED osmolarity thresholds. The output may depend on the sampling method or on the instrument[67]. Moreover, the sample's electrical impedance is temperature-dependent, thus impedance-based osmometry can be biased if the temperature is not controlled.

This measure, assessed with the impedance-based osmometer was added to the protocol of ***the longitudinal study of biomarkers' trends*** in ***Chapter III*** of this dissertation and was compared with the ocular measures assessed with the proposed experimental techniques, with emphasis put on its potential correlation with tear volume, tear clearance and tear stability.

1.3. Ocular surface inflammation

Ocular surface inflammation is included in the definition of DED. The quantifiers of ocular inflammation, recognized in a research settings, are not specific for DED and the protein level method and multiplex cytokine systems analysis, recently more commonly used, bare multiple technical difficulties, that impede their clinical application[75]. Currently, most practitioners do not include tests for inflammation as a clinical marker of DED. Development of more clinically utile techniques to assess tear film protein level is beyond the scope of this dissertation. The most commonly measured clinical sign that is suggestive of ocular surface inflammation and could be easily assessed in a clinical setting is the severity of conjunctival redness[76-78]. It is a consistent sign of conjunctival vascular dilatation and reactive change to pathological stimuli. It can occur in any disease with inflammation, including DED. It can be observed and graded during slit-lamp examination.

In this dissertation, an objective measure of ocular redness was used. Bulbar and limbal redness were measured based on images automatically scored with K5M. The ocular redness index within the K5M software makes measuring bulbar redness easy to perform in a clinical setting and provides additional measure of ocular surface inflammation[78].

1.4. Ocular surface damage

Other ocular dysfunction that has been identified as one of the core ocular sings of DED and was included in its definition, is the *ocular surface damage*. Punctate staining of the ocular surface is characteristic to many ocular diseases and its distribution, pattern and severity may provide a clue on the aetiology of these diseases[79]. Fluorescein staining occurs whenever viable cells experience a compromise, e.g., a disruption in superficial cell tight junctions or defective glycocalyx[79,80], while lissamine green stains epithelial cells only if the cell membrane is damaged[81-83]. Sequential staining or using more than one paper strip increases the likelihood of observing ocular surface damage in a form of ocular surface staining[84,85], thus, several studies using mixtures of two dyes for simultaneous staining of the cornea and conjunctiva have been proposed[84,86,87]. The mixture of 2% fluorescein and 1% lissamine green found to be optimal in terms of comfort and staining efficacy[86].

A battery of grading systems is used for the recording of staining severity[51,88-92]. In this dissertation, the Efron's scale was used to score staining of the cornea and conjunctiva[93] and Korb's scale[94,95] was used to grade the level of lid wiper epitheliopathy (LWE). Corneal and conjunctival staining have been shown to be informative markers of the disease in severe manifestation of DED, however they showed poor correlation with the disease severity in mild/moderate cases[9]. In this dissertation, grading of the corneal and conjunctival staining was used mostly for sanity check, rather than as a potential biomarker. In order to disengage from the study the subjects, who had experienced adverse effects of contact lens wear, it was necessary to use some screening criteria which would effectively highlight corneal, conjunctival and eyelid tissue damage[84,87,96,97].

The most recommended method, which was also used in this dissertation, is to combine fluorescein and lissamine green-based staining with the use of moistened florets.

1.5. Tear turnover rate

As hypothesized in the introduction, the *loss of homeostasis of the tear film*, which describes the fundamental process in DED pathogenesis, may be expressed by a lack of equilibrium between hydrodynamic processes occurring in the tear fluid. Tear turnover has been described as a global physiological measure of the integrity of the lacrimal system and tear exchange on the ocular surface[45,98,99]. The tear turnover rate (TTR), a temporal measure of tear turnover is proportional to the summation of effects of tear secretion by the glands (denoted by S), tear fluid transudation through the conjunctiva (C), tear drainage through the nasolacrimal duct (D), tear evaporation (E) and conjunctival (PC) and corneal (PK) permeability to tears, hence it can be described as:

$$\mathrm{TTR} \sim (\mathrm{S} + \mathrm{C}) - (\mathrm{D} + \mathrm{E} + \mathrm{PC} + \mathrm{PK}) \qquad \textbf{(1)}$$

Where '∼' denotes proportionality. In normal circumstances D > E > PC > PK.

One can see that TTR considers most of the hydrodynamic processes that occur in the tear fluid, thus it is multifactorial in nature. Balance between components constituting equation **(1)** may be disrupted in a variety of ways, leading to loss of tear film's homeostasis. DED can manifest itself, e.g. as an increase in tear film evaporation, decrease in aqueous tear production or as an increase in the ocular surface permeability to various substances by compromised surface epithelium. Moreover, DED is sometimes diagnosed as a mixture of both evaporative and aqueous deficient in aetiology or can have iatrogenic or neuropathic nature. Thus, it was hypothesized that the ocular measure that

considers all hydrodynamic processes occurring in the tear fluid may prove to be a good singular macro-type biomarker of DED. The delay in tear turnover is used as a sign of impaired LFU's integrity. The marker is multifactorial and has been shown to correlate with many DED-related ocular pathologies. Figure 1 schematizes the literature review on the factors associated with delayed tear turnover.

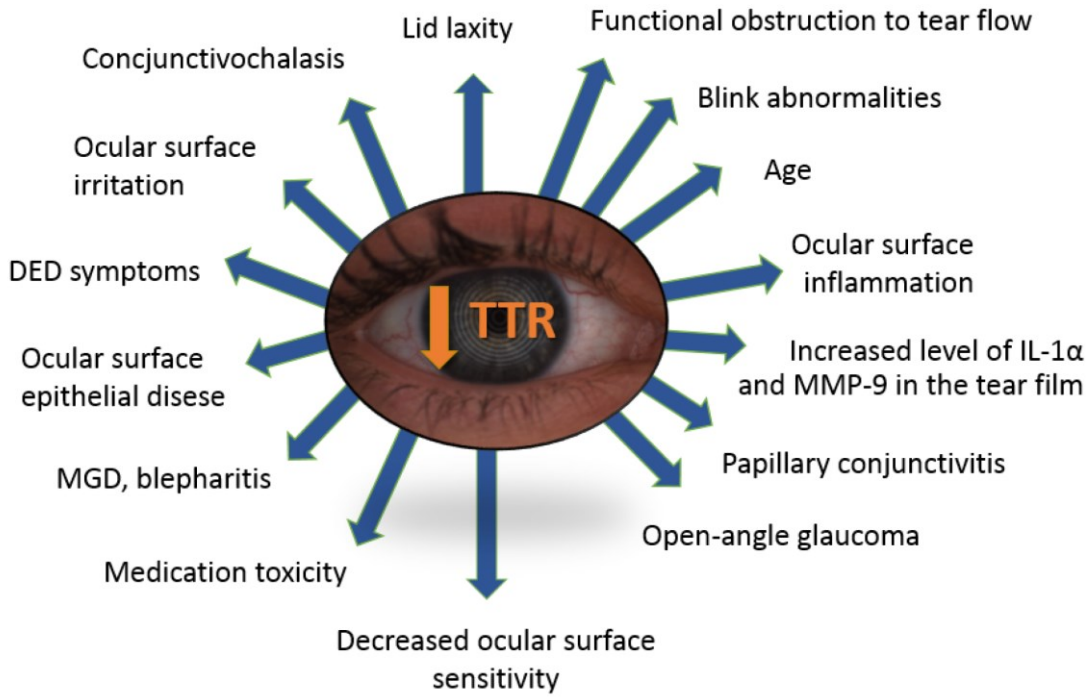


Figure 1. The overview of factors associated with decreased tear turnover or tear clearance

TTR has been extensively studied after the development of commercially available fluorophotometer in the 80's and has shown its potential in DED diagnosis. However, because of its sophistication, the most popular technique of TTR assessment - fluorophotometry, was mostly confined to research settings and was overlooked as a clinical measure of DED[16]. In addition, simple alternatives to TTR, developed to be

used in clinical settings, are, at best, semi-quantitative, qualitative or subjective and do not allow observation of temporal tear film characteristics.

In ***Chapter II***, two clinically applicable alternatives for TTR estimation were proposed. As the motivation behind development of these two techniques cannot be fully appreciated without an introduction of the state-of-the-art methodologies and their limitations, the paragraphs below are to summarize the literature review on tear turnover assessment and quantification.

Originally, tear turnover was described as a physiological measure of the integrity of the lacrimal system[45,98,99]. Tear clearance rate (TCR) refers to the same phenomena observed by other means than fluorophotometry. TTR is described in literature as an indirect measure of DED-associated ocular surface irritation[100-103] and is reduced in symptomatic DED subjects[45,99,104-106]. It correlates with the severity of ocular epithelial disease assessed with fluorescein staining[102,103,107] rather than with the reduced aqueous tear production assessed with Schirmer test[107]. Tear clearance delay was associated with Meibomian glands dysfunction (MGD)[102,103] and ocular surface hypoesthesia[98,101-103,108-111]. Additionally, age and age-related factors, e.g. conjunctivochalasis, lid laxity, functional obstruction to tear flow and blink abnormalities may all impair tear turnover[101,102,112-114], however, some studies showed no relation of TTR with age[115-117]. Reduced tear clearance promotes ocular surface inflammation[101], as it leads to accumulation of cytokine interleukin-1α and the activity of matrix metalloproteinase and gelatinase B in tears[101-103,108,109,118]. TCR was shown to improve with topical methylprednisolone treatment, together with decrease in ocular irritation symptoms, in conjunctival redness and the level of surface epithelial disease[101,102]. Additionally, delay

in tear turnover may lead to prolonged exposure to topical medications and their preservatives (like benzalkonium chloride) on the ocular surface compared with healthy subjects, therefore the affected individuals have a higher chance to develop ocular surface medication toxicity[99,101,102,119]. Tear clearance is also reduced in subjects with contact lens associated papillary conjunctivitis[120].

Delayed tear clearance could become one of the best measures for identifying patients with tear film disorders that respond to anti-inflammatory therapy[98]. TTR values reported for DED groups are two to five times lower than the ones reported for controls[105,117]. The meta-analysis conducted by Tomlinson et al. has shown a 50% reduction in TTR in aqueous deficient DED and 25% reduction in evaporative manifestation of DED with respect to healthy subjects[121], thus TTR may potentially be used to aid distinguishing between DED subtypes[68]. Large variability of the reported results can be due to different groups of subjects and different diagnostic criteria used to classify them[10]. Reported group means of TTRs have also relatively large reported standard deviations, suggesting large variability among subjects.

Generally, TTR and TCR are estimated based on direct or indirect observation of the elution of dye in the tear fluid over time post-instillation and with the production and elimination of new tear fluid. The general approach is to follow the elution of a tracer molecule from the tear film or tear menisci with the use of electromagnetic spectrum detectors[45,99,105,116,117,122-130]. This family of methods include: the most popular – fluorophotometry and lacrimal gamma scintigraphy (or scintillography)[45,131-136]. TTR assessment with fluorophotometry was described as one of the additional measures that could be used to diagnose and monitor DED and addressed the need to develop

cheaper, shorter and simpler methodologies for its quantification. Since the publication of DEWS I, no studies have been conducted to address this issue. Thus, fluorophotometry is still considered the gold standard in TTR and tear flow assessment. In Figure 2 a schematic representation of a basic fluorophotometer is shown.

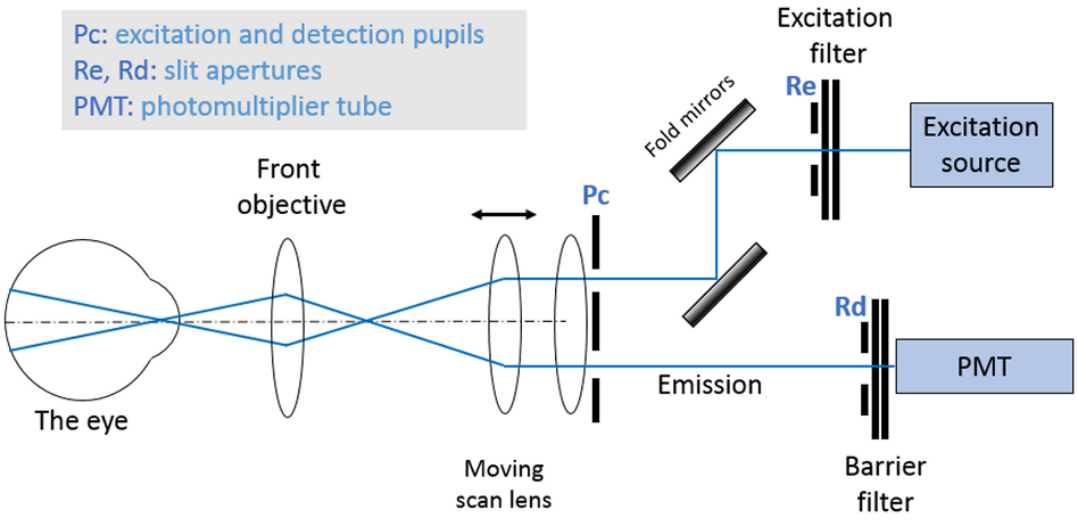


Figure 2. Schematic diagram of a basic fluorophotometer for in vivo assessment of TTR[16]

Fluorophotometer utilizes a beam of light that passes through an excitation filter (transmittance: 430-490 nm) to excite the transparent layers of the eye. The light emitted from these ocular tissues passes through the barrier filter (transmittance: 510-630 nm) and the level of fluorescence is measured by a photomultiplier, where the excitation and emission beams reach, passing through the same lens system, coming from the area called the *focal diamond*. The anterior segment attachment must be used to reach the area of interest. This is possible because the focal diamond can be moved along the optical axis of the eye, by moving the lens system with a stepped motor. Disodium fluorescein is the most commonly used fluorophore in TTR assessment. It is a hydrophilic molecule characterised by low topical toxicity and with excitation wavelengths of 475–490 nm and emission of 510–520 nm[99].

Studies report several attempts to standardize the procedure of TTR assessment and the development of a custom-written software for data processing[116]. The standardized procedure lasts up to 30 minutes[110,116] during which fluorophotometric scans are performed every two minutes with a commercially available fluorophotometer designed for in vivo analysis (Fluorotron Master, Coherent Radiation Inc. Mountain View, CA, USA). This is performed following an instillation of 1μL of 2% sodium fluorescein into the subject's conjunctival sac with a micropipette. There is a variation among studies in terms of used fluorescein volume (from 1 to 5 μL), fluorescein concentration of the solution (up to 10%), sampling rate of the device and the duration of the measuring part of the procedure (10 – 30 min). In vivo fluorophotometric measurements can be performed on the tear meniscus or pre-corneal tear film, however they are always restricted to a small area within the focal diamond. Continuous fluorophotometric measurements have also been performed[122].

Kinetic studies of TTR with fluorophotometry illustrate the biphasic nature of tear dynamics[45,99,116,122,137,138]. Two phases of tear turnover can be distinguished based on the observation of fluorescence intensity decay in the tear film after topical instillation. An incipient, rapid phase of tear clearance is presumed to be caused by reflex lacrimation in response to topical instillation[45,99,101,112,136,138]. This 'reflex' phase lasts approximately five minutes post-instillation and varies from subject to subject. It is characterized by a rapid decay in fluorescence intensity over time. The scientific observations related to this phenomenon suggest its 'reflex' nature. The rate of this rapid phase is correlated with subjective signs of irritation at the time of instillation[138] and can be suppressed with anesthetics[101,112]. Its level is also highly dependent on blinking rate[112,122,136,139].

Elderly subjects, especially women, tend to lose this initial phase of tear clearance and were reported to have generally lower TTRs[114,138,140].

The second, slower phase of tear turnover presumably represents basal conditions of secretion and is used to calculate TTR[99,116,123]. This basal part of the fluorescence intensity curve is fitted with an appropriate software[116] and the decay in fluorescence is calculated from the logarithm of the curve obtained from the formula below:

$$\mathrm{TTR}(T_0) = \frac{100\,[C_T(T_O) - C_T(T_O+1)]}{C_T(T_O)}\left[\frac{\%}{\mathrm{Min}}\right], \quad \textbf{(2)}$$

where $C_T(T)$ Represents the fluorescein concentration in the tear film at time T (min), while T_0 represents any given moment after instillation. Assuming a monophasic decay of fluorescence after initial five minutes with a decay time constant $\beta(\mathrm{min}^{-1})$, the fluorescein concentration can be described with the following formula:

$$C_T(T) = C_T(T_0)e^{Bt}\left[\frac{\mathrm{Ng}}{\mathrm{Ml}}\right]. \quad \textbf{(3)}$$

The tear turnover rate $\mathrm{TTR}(T_0)$, which is described as percentage drop in fluorescein intensity decay per minute is then described as:

$$\mathrm{TTR}(T_0) = 100\,(1 - e^{Bt})\left[\frac{\%}{\mathrm{min}}\right]. \quad \textbf{(4)}$$

Table 1 provides several TTRs assessed by means of in vivo fluorophotometry that were reported in literature.

Table 1. Group values (means ± standard deviations) of tear turnover rates measured in vivo with fluorophotometry, reported in literature

Year	Report	Type (number of subjects)	Reported TTR (Mean ± SD)
2001	Tomlinson et al.[141]	Normal (20)	21.4 ± 11.1
2001	Tomlinson et al.[62]	Normal (9)	16.6 ± 6.7
2001	Sorbara et al.[104]	Normal (10) Symptomatic DED (10)	11.85 ± 3.31 4.89 ± 2.75
2001	Pearce et al.[126]	Normal (56) Normal (49)	12.65 ± 6.64 17.78 ± 6.34 (New method)
2002	Keijser et al.[142]	Normal (16)	14.3 ± 6.5
2003	Mcculley et al.[143]	Normal (22) DED (35)	16.3 ± 7.3 13.5 ± 9.3
2004	Sorbara et al.[104]	Symptomatic DED (10) Asymptomatic DED (10)	4.89 ± 2.74 11.85 ± 3.31
2005	Khanal, Tomlinson[45]	DED (8) MGD (6)	8 ± 3 11 ± 6
2008	Mccann et al.[144]	Normal (15)	20.6 ± 9.3
2009	Tomlinson et al.[121] (Meta-analysis)	Normal (187) DED (197) Aqueous deficient DE (83) Evaporative DED (94)	16.19 ± 5.1 9.26 ± 5.08 7.71 ± 1.02 11.95 ± 4.25
2010	Khanal et al.[68]	Normal Aqueous deficient DED Evaporative DED	15.24 ± 5.69 5.74 ± 3.69 12.61 ± 7.56

DED – Dry eye disease; MGD – Meibomian Gland Dysfunction; SD - standard deviation.
Source: Garaszczuk et al.[16]

Large variations in tear film production in DED subjects and differences between DED and normal subjects in basal tear volume[145,146] have been reported. The tear turnover rate was found to increase suddenly due to a variety of stimuli like coughing, sneezing, wind or even psychological factors like an effort to keep the eyes open[138] or variations in blinking rate in some individuals[123]. Additionally, some researchers suggest that TTR follows a circadian rhythm. This claim is strongly supported by the fact that TTR values reported in the morning are significantly higher compared to those reported later throughout the day[66,122,124,147].

Methods used to asses TTR and TCR have their well-studied limitations. Golden standard fluorophotometry-based method requires considerable skill and extensive period of time to obtain results (from 10 up to 30 minutes[116,126]). Methodologies that allow dynamic, quantitative measurements require expensive, specialized equipment. Lacrimal scintigraphy provides a visual evidence for tear drainage; however, it is a technique that is invasive and relatively expensive. Moreover, it requires radioactive substances to be used. Moreover, the focal diamond of a basic fluorophotometer is 50 μm wide, 1.9 mm high and around 0.5 mm deep. Its depth approximates tear film and corneal thickness, making it difficult to determine whether the readings are coming from the tear film or the substantial portion of corneal tissue. This limits the spatial resolution of the device. In some individuals, especially DED subjects, the precorneal tear film may break-up rapidly, exposing the cornea. Additionally, compromised corneal epithelium of these subjects can be excessively permeable to sodium fluorescein, increasing inhibited corneal fluorescence over time[99]. Hence, it is sometimes desirable to perform the assessment of TTR on the collected tear samples (in vitro)[66,102,103,109,110].

Fluorescein tear clearance tests (FCTs) are simpler and less time-consuming alternatives to fluorophotometry and scintigraphy. They were developed to facilitate clinical application of TCR assessment. FCT rates or scores can be estimated based on the observation of how quickly the dye appears in the nasal cavity[148,149], based on direct visualization of fluorescein drainage with an endoscope[150] or by comparing the colour of the dye with visual semi-quantitative scale[151-153]. Until recently, only few FCTs were developed[98,100,101,107,109,110,123,154]. Two main limitations of these methods are evident: they are most commonly based on subjective assessment and fail to follow dynamic changes occurring in the tear fluid.

Corneal permeability to sodium fluorescein, especially in subjects with compromised corneal epithelium may influence fluorophotometric assessment of TTR[99,122,130,137,155-157]. After 30-40 minutes post instillation the corneal fluorescence starts to influence the results and the readings show significantly lower rate of decay than the one characteristic to basal tear flow[99,122,137]. To address this limitation, Joshi et al. Reported a novel technique for sequential measurements of TTR and corneal epithelial permeability to fluorescein[155]. They have determined that 2 μL of 0.75% fluorescein concentration provides the most reproducible estimates. Studies suggest that corneal permeability to fluorescein decreases in DED subjects undergoing treatment with unpreserved artificial tears, because of the corneal epithelium restoration[156-158]. This effect is counteracted by preservatives[157-159]. Additionally, the inherent auto-fluorescence of the cornea must be considered by subtracting its rate from the measured fluorescence intensity. Corneal auto-fluorescence was shown to increase with age[160].

Attempts have been made to improve the precision and speed of basal TTR assessment. Pearce et al. were investigating a minimum time required for TTR estimation, while using their improved automatic fluorophotometry-based method. This technique incorporates six measurements in a total of 10 minutes to obtain reliable TTR estimation[126].

Also, the time necessary to take a single measurement may contribute to inaccurate TTR estimations and reflex tearing. Scans of the tear film, anterior eye, anterior chamber and crystalline lens last approximately 20 seconds, however research suggests that this time can be shortened to 8 seconds[161-163] for TTR measurements. Fluorophotometric scans must be made at a consistent and fixed time after the blink. On the other hand, fixed blinking rate would ultimately lead to errors in TTR estimation, since the natural blinks differ in characteristic from the forced ones[164,165]. Doane showed that a significant portion of the Bell's movement can be observed during a forced blink, while during normal, spontaneous bink no rotation of the globe occurs[164]. The voluntary blinks are usually more complete and more uniform in quality than the spontaneous ones[166]. Due to these factors, the TTR assessment has been mostly confined to research settings. Although simpler alternatives developed for clinical application are inexpensive and less time-consuming, they are not direct, often subjective[153] and neither they allow following temporal tear film characteristics nor quantify fluorescein concentration. It was also reported that in more than 20% of normal subjects the topically instilled dye cannot be recovered in the nasal cavity[151]. Maurice[167] and Doane[168] showed that low volumes of tears drawn into the drainage system during normal, non-reflex tear exchange can be absorbed by mucosal surface of the nasolacrimal duct.

Summarizing, new studies show that there is still room for improvement in the field of TTR assessment and the need to develop more clinically applicable, objective and less time-consuming methodologies. New approaches that were proposed in ***Chapter II*** include measuring tear meniscus morphology dynamics with OCT[169-171] and the analysis of tear exchange by means of fluorescein profilometry[172].

1.6. Tear meniscus morphology

The major part of the tear fluid is contained within the two menisci[173], therefore they serve as reservoirs of tears[174]. Tear menisci are formed by the tears lying at the junctions of the bulbar conjunctiva and the margins of both the upper and lower eyelids. The quantitative assessment of tear menisci (meniscometry) is used for tear volume estimation as the most preferred method. Inferior tear meniscus height (TMH) is used as a marker of muco-aqueous tear volume[175,176] and was shown to be linearly proportional to the lacrimal secretory rate[138]. Meniscometry have a wide range of applications[176-179] and can be used to aid DED diagnosis[176,180-182]. The average normal TMH ranges from about 100 to 600 μm with the mean value of approximately 250 μm[174,180,183,184]. Both superior and inferior menisci were estimated to have roughly equal size under normal conditions[174], however in some circumstances, e.g. after topical instillation the inferior TMH is significantly larger than the superior TMH[185].

It is worth noting, that none of the geometrical parameters describing the tear menisci corresponds to the central pre-corneal tear film thickness[174]. It is well established, that shortly after each blink, the pre-corneal tear film becomes physically isolated from the tear menisci, such that the diffusion between these two tear compartments does not

occur[186,187]. This can be observed as a black line at the ocular margins in fluorescein-stained tears, as shown in the Figure 3.

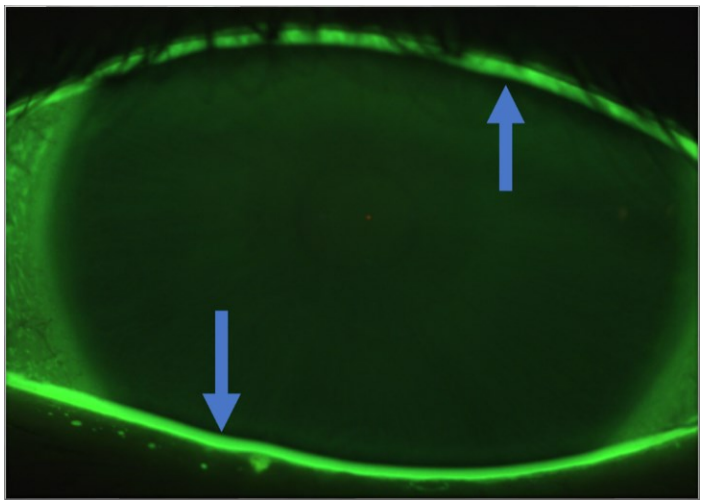


Figure 3. Black-line formation between tear menisci and tear film in fluorescein-stained tears. Image obtained for one of the subjects with K5M. Arrows delineate the location of the thin black lines separating the tear film from the tear menisci

Meniscometry can be performed with the use of a slit-lamp biomicroscopy[188,189] or with OCT[177,185,190,191]. The simplest, *en face* method of tear meniscus visualization is by far the most commonly applied in clinical setting. Meniscometry measures correlate with other DED tests and have relatively good accuracy[180,192]. However, the slit lamp approach is operator-dependent and has important limitations related to rapid changes of tear meniscus parameters after the blink. Additionally, this method is characterized by a poor inter-visit repeatability[26]. Additionally, meniscometry can be influenced by locus along the lid margin, time of day, temperature, humidity, air speed, and illumination[193-195].

The OCT-based assessment of the tear meniscus has been extensively studied in the last decade[176,177,190,196-212] with the upper and lower TMH, tear meniscus area (TMA), tear

meniscus radius of curvature and tear meniscus depth (TMD) being the most commonly studied parameters. Spectral-domain OCT-based meniscometry has shown good intra-observer and inter-observer repeatability[201,206,210], superior to the ones characteristic to time-domain OCT-based meniscometry[202,212]. OCT ensures relatively good repeatability and allows observation of dynamic changes of tear meniscus morphology during blinking[185] and after topical instillation[184,213-215]. The OCT-based measurements are instrument-dependent[177,202], and can be influenced by conjunctivochalasis, disorders of lid margin congruity, and apposition between the lid and ocular surface[190,216]. The main advantage of the OCT-based meniscometry is that it provides a non-invasive, rapid, simple and more in-depth visualization of both superior and inferior tear menisci[177,185,190,191]. However, the analysis of the acquired images may be complex, time-consuming and operator-dependent[210]. Thus, the software allowing dynamic image analysis is needed to minimize interfering factors related to eye movements. Such software was developed by Bartuzel et al.[200] in MATBLAB and was used in this dissertation to study the OCT-based meniscometry (***Experiment 2***). Results of the experiments showing the performance of the implemented algorithm are reported in ***Chapter II.*** Additionally, this algorithm was used to calculate TMH, TMD, TMA and TCR in ***the longitudinal study of biomarkers' trends.***

CHAPTER II. NEWLY DEVELOPED EXPERIMENTAL TECHNIQUES

Following chapter describes the experimental techniques that can be used to follow, analyse and quantify different aspects of tear dynamics. Temporal measures of these dynamic changes were proposed as new markers of LFUs integrity and tear exchange on the ocular surface and could become the new macro-type biomarkers to support DED diagnosis. Considering the potential applicability of TTR and TCR measurements in DED diagnosis, summarized in ***Chapter I***, it is of interest to develop clinically applicable methodologies for their quantification. ***Chapter II*** proposes such new methods and algorithms in a form of three separate experiments.

EXPERIMENT 1. QUALITATIVE ASSESSMENT OF TEAR EXCHANGE ON THE OCULAR SURFACE BY MEANS OF FLUORESCEIN PROFILOMETRY

The main goal of this experiment was to introduce new, clinically applicable method for TTR estimation by means of corneo-scleral fluorescein profilometry. The principal focus was put on making the method objective and free from aforementioned limitations of other devices used for TTR assessment. The methodology described below was inspired by fluorophotometry, however, as it differs in terms of the utilized device and temporal characteristics of the observed phenomena, phrase *TTR* was substituted with the *Tear Fluorescein Washout Rate (TFWR)* when referring to the results of profilometry-based technique analysis. The fluorescein profilometer - Eye Surface Profiler (ESP, Eaglet Eye B.V., The Netherlands) was developed for topographical measurements of the whole exposed corneo-scleral surface. This device projects two grids with blue light at two different angles to create a diffusely emitted pattern on the ocular surface. Fluorescein must be added to the tear film to visualise this pattern. Radiation emitted by the excited fluorescent marker passes through a built-in yellow filter and is subsequently captured by a fast CMOS camera. A single image of the diffused pattern obtained with the ESP for one of the subjects is shown in Figure 4. Unlike the standard methodologies for TTR analysis, fluorescein profilometry is not limited to a small portion of the ocular surface and allows following tear fluorescence decay on the entire exposed ocular surface. Additionally, it seems to be unaffected by the corneal permeability to sodium fluorescein, as the diffused image cannot be observed without fluorescein being instilled or after being washed away from the ocular surface.

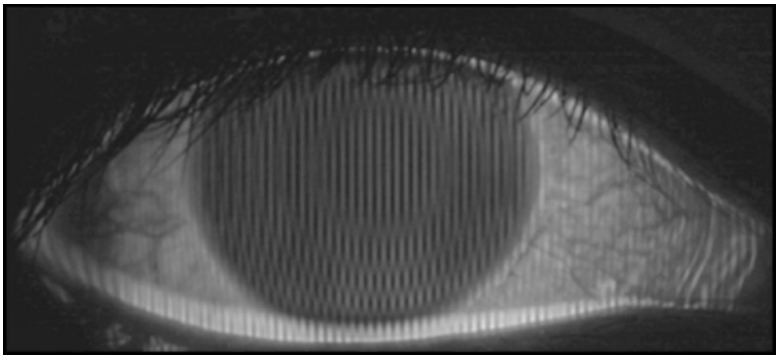


Figure 4. An exemplary image of the diffused pattern acquired with ESP for one of the subjects

E1.1. Methodology

Study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants after the nature and possible consequences of the study were explained. Young, healthy subjects were recruited. Exclusion criteria were consistent with the study reported in ***Chapter III***, including signs and symptoms of severe eye dryness or inflammation, recovery after general or ocular surgery, refractive procedure or any observable tear flow impairment, systemic disease and the use of medications known to influence the ocular surface or tear film quality. Subjects were advised to cease instilling any topical solutions at least a day and refrain from wearing their habitual contact lenses at least three days before commencing the study. For precaution, to minimize the risk connected with the use of vital dyes, all subject prone to any type of allergic reaction or with reported adverse reaction to topically or intravenously administered fluorescein were excluded from participation in the experiments.

Forty subjects (24F/16M) aged (mean ± standard deviation) 32 ± 14 y/o (ranged from 21 to 70 y/o) were chosen from the group of volunteers. The study protocol consisted of the review of medical history, McMonnies questionnaire (McMQ)[217] (see ***Appendix 1***) and slit

lamp examination, including the assessment of eyelids, ocular adnexa and anterior eye surface for signs of irritation, tear flow impairment and lid-parallel conjunctival folds, TMH, blinking rate estimation and FBUT. The image intensity video recording by means of fluorescein profilometry was performed before FBUT estimation and was followed by a 10-minute break. The temperature and relative humidity in the laboratory were stable and monitored with a thermo-hygrometry device (C3121, Comet, Czech Republic).

Data on the circadian rhythm of TTR are equivocal, however following the evidence that tear exchange rate may vary with the daytime[124], measurements were performed in the morning.

E1.2. Image acquisition and data analysis

Only one out of two projecting ESP diodes was used in order to minimize the effect of reflex tearing caused by excessive radiation[218]. ESP was used in an unconventional way to observe dynamic changes occurring in the tear film on the exposed ocular surface after fluorescein instillation. Instead of acquiring an single image, as the topographical measurement usually requires, the projected pattern was recorded in a series of images captured with a fast CMOS camera. The best measurement practice to assess corneo-scleral topography with the ESP has been previously described[218]. Summarizing, for the device to deliver accurate and repeatable measurements of the anterior eye surface, two important factors must be considered:

- the instrument must be situated at the optimal acquisition position with fixation spots and illumination spots aligned;

- the anterior eye surface must be uniformly covered with a mixture of tear film and fluorescein.

The optimal position for ESP image acquisition corresponds to the near (to the operator) end of the instrument's depth of focus. ESP is equipped with three focus aiding tools to guide the operator to this location. Thus, the measuring procedure to capture the intensity decay video consists of:

- positioning the subjects and adjusting the chinrest,
- aligning the instrument's centration cross with the geometrical centre of the cornea with the help of the centration tool,
- bringing the instrument to the range of depth of focus with the help of the optical focusing tool,
- setting the instrument at the near end of the depth of focus range,
- blocking the instrument in the obtained position, to prevent defocus,
- instilling fluorescein without subjects moving their head away from the device, (Subject should be asked to blink gently three times to evenly distribute the dye and fixate on a target cross until advised otherwise),
- if necessary, small adjustments of focus can be performed post-instillation,
- subsequently, the blue light is being turned on, recording started and continued until the projected image is barely visible in the tear film.

The acquisition is performed in mesopic conditions for increased image contrast. While using ESP to acquire images of the diffused pattern, care should be taken to instil enough fluorescein to achieve good contrast of the diffused image. It is also crucial to avoid the nonconfluent distribution of the dye in the tear film, because it may distort the pattern,

ultimately leading to errors in TFWR estimation. An exemplary image of the proper distribution pattern, acquired for one of the subjects was displayed in the Figure 5A.

On the other hand, the eye should not be flooded with fluid and care should be taken to not to induce reflex lacrimation, as this will lead to tear pooling and uneven distribution of dye in the tear film, as shown in the Figure 5B.

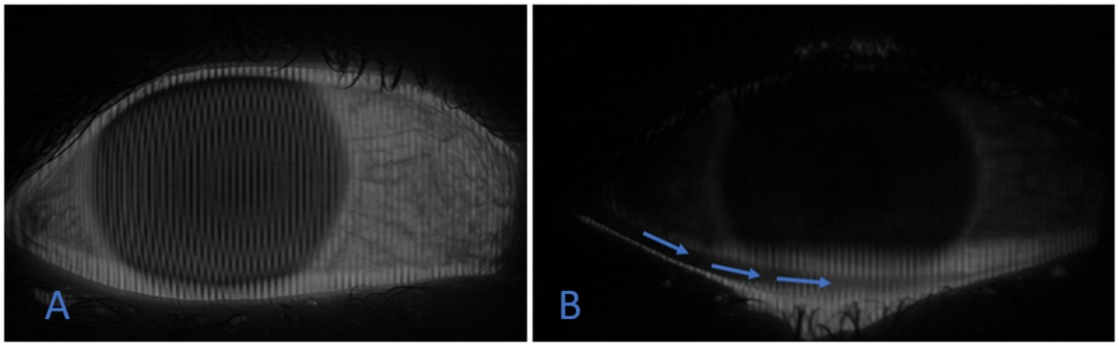


Figure 5. Exemplary images acquired with ESP with A: well distributed fluorescent dye on the ocular surface or B: with visible tear pooling and the flow of tears observed after blink (marked with blue arrows)

As the fluorescein solution designed for topical instillation is poorly accessible in some European countries, including Spain and Poland, the method of fluorescein application with a sterile fluorescein strip was employed in this experiment. Considering this, care should be taken to maintain the amount of fluid as constant as possible and to maintain the method of application standardized. Without standardization, this experimental method cannot be considered quantitative. The proposed methodology would be the one adapted for FBUT estimation, where several measurements are performed, and the results are averaged to obtain a reliable estimate.

A drop of lubricating ophthalmic solution of 0.1% sodium hyaluronate (Hylo-Parin, Ursa Pharm, Germany) was applied from a dedicated container. This drop was used to moisten

the fluorescein sodium 1 mg ophthalmic sterile strips (Bioglo, HUB Pharmaceuticals, CA). The strip was applied lightly to subjects' lower bulbar conjunctiva. The container allows the application of repeatable volume of the ophthalmic solution (3 μL) with each instillation. The method of fluorescein application with the strip provides superior sterility over the topically instilled solutions.

A drop of fluid was dropped under the force of gravity, without additional shaking of the container or the strip. This way the fluorescein was applied in the most standardized manner possible in this laboratory setting, so that repeatability of the measurements was maximized. The diffused image intensity is proportional to the amount of fluorescent dye in the tear film. The projection is visible for as long as the fluorescein is present in the subjects' tear film. While subjects were instructed to focus on the instrument's fixating cross and to blink freely, the recording of the pattern through an in-built yellow filter with a CMOS camera allowed observation of the fluorescein image intensity decay. As can be seen in the Figure 6, the dye was gradually replaced with new tears and the image intensity was decreasing post instillation. Since the recording was acquired with extremely high-resolution and a 20-second acquisition was consuming as much as 2 GB of computer memory, the recording was kept as short as possible and stopped when projected image was barely visible or, in cases where the tear wash-out was delayed, when an apparent difference in image intensity was observed. The approximate acquisition time was up to one minute. The recordings, considering their quality and size, could not be viewed with any conventional computer video software.

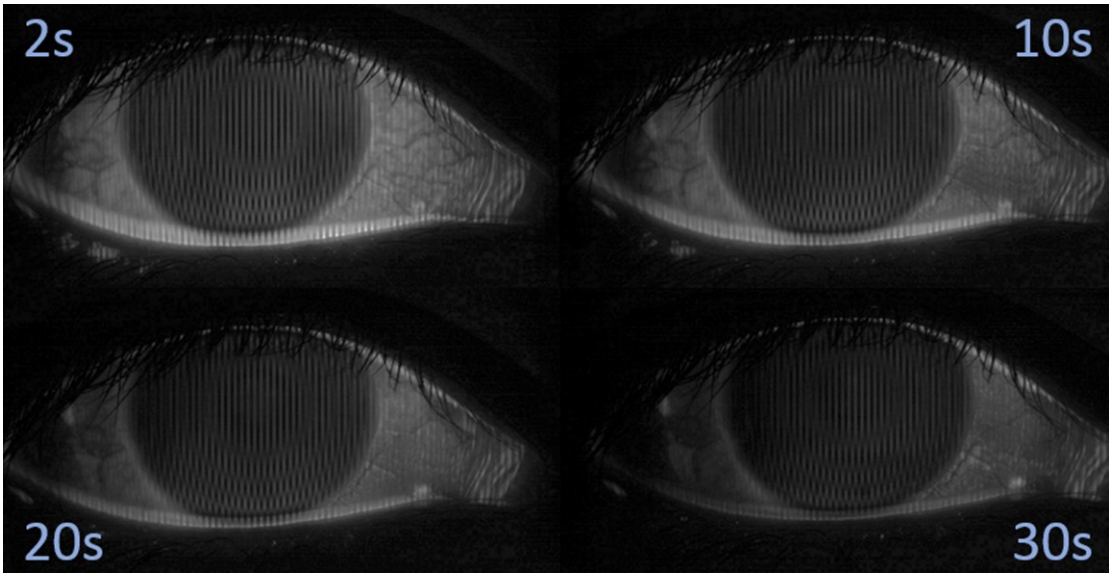


Figure 6. Stages of the diffused image fluorescence intensity decay, acquired for one of the subjects with ESP

The fluorescence intensity decay curve was obtained using a custom-written MATLAB software, which calculates the mean intensity of the image within the area that corresponds to the exposed anterior eye surface for every frame of the captured video (as shown in the Figure 7).

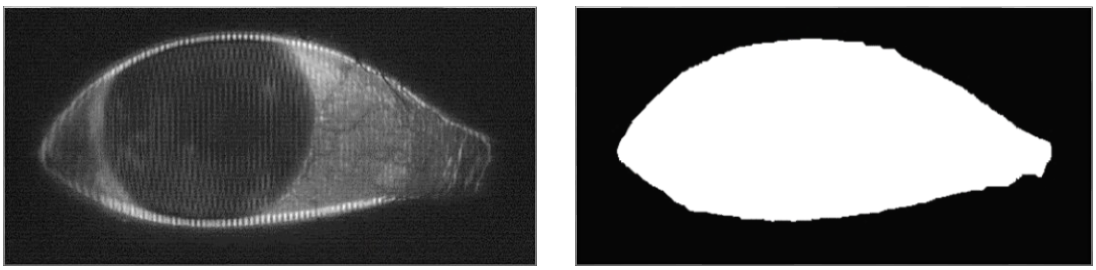


Figure 7. Left: An illustrative frame from the ESP video sequence acquired for one of the subjects; right: Demarcated area of analysis corresponding to this image

The two main steps to estimate the fluorescence intensity decay curve are as follows:

- generation and application of the mask (Figure 7) that defines the area of analysis;

- calculation of mean image intensity in the determined area of analysis

The shape and position of the mask can be changed, however in this experiment the whole exposed ocular surface was covered. The monophasic exponential model of the image intensity I(T) decay in time T was assumed,

$$\mathrm{I(T)} = \mathrm{A}e^{-\beta \mathrm{T}}. \quad \textbf{(5)}$$

After removal of the signal artefact due to blinks, the amplitude A and the decay constant β *w*ere estimated using the linear least-squares procedure by taking first the logarithm of the model, that is:

$$\log(\mathrm{I\ (T)}) = \log(\mathrm{A}) - \beta \mathrm{T}. \quad \textbf{(6)}$$

Then, a time varying TFWR was defined as:

$$\mathrm{TFWR(T)} = \frac{\mathrm{I(T)}}{\mathrm{I(0)} \times \mathrm{T}} 100 \left[\frac{\%}{\mathrm{min}}\right]. \quad \textbf{(7)}$$

The TFWR is expressed as a percentage drop of the mean masked image intensity after time T = 0.5 min. The period needed to record the video and assess TFWR was set arbitrarily reflecting the pragmatic aspects of the data acquisition. The 30-second margin was chosen, considering the duration of the shortest recording. However, the exponential decay profiles can be provided for the entire phase of the observation. An exemplary intensity decay curve acquired for one of the subjects and processed with the software is

displayed in the Figure 8. Additionally, OCT-based estimation of TCR (as proposed in ***Experiment 2***) uses the same margin, so that these two methods are easier to compare.

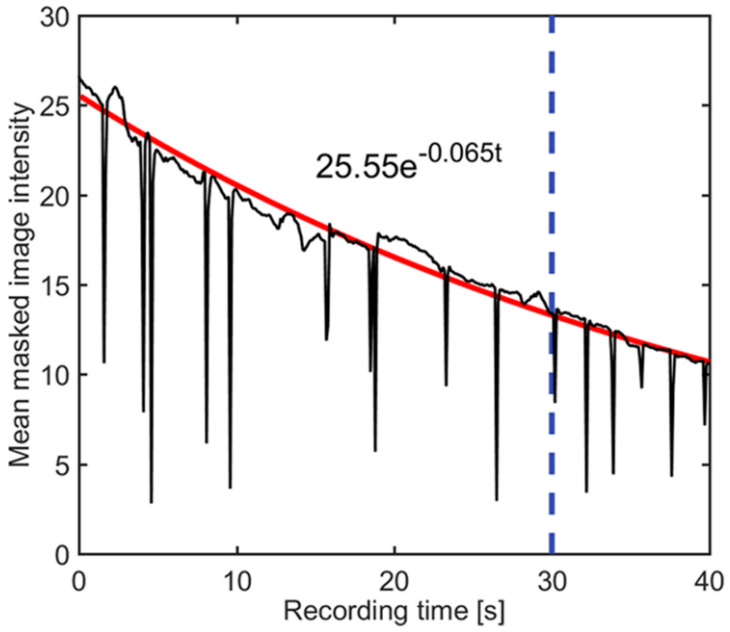


Figure 8. An exemplary curve of mean masked image fluorescence intensity decay acquired for one of the subjects (black line), with 30 seconds margin marked (grey dashed line) and fitted curve (red line), t - time

E1.3. Results

It should be noted that the sample size was not estimated before commencing the study, as no reasonable information regarding parameter variability was available. However, post-hoc analysis, conducted for 90% power at the 5% significance level, found that the chosen sample could assess differences of about 11% for profilometry-based TFWR at 30-second margin. The temperature and relative humidity in the laboratory were stable and monitored and averaged around (mean ± standard deviation) 24.6 ± 1.4 ºC and 26.1 ± 4.4 %RH, respectively. For all subjects, the mean TFWR was estimated as 39 ± 23% at 30-second margin, ranging from 2% to 83%. The TFWR distribution was tested for normality and the null hypothesis was not rejected (Lilliefors test, P = 0.310). Figure 9 shows correlation plots between TFWR and other ocular measures for which the statistically significant linear correlations with TFWR were noted.

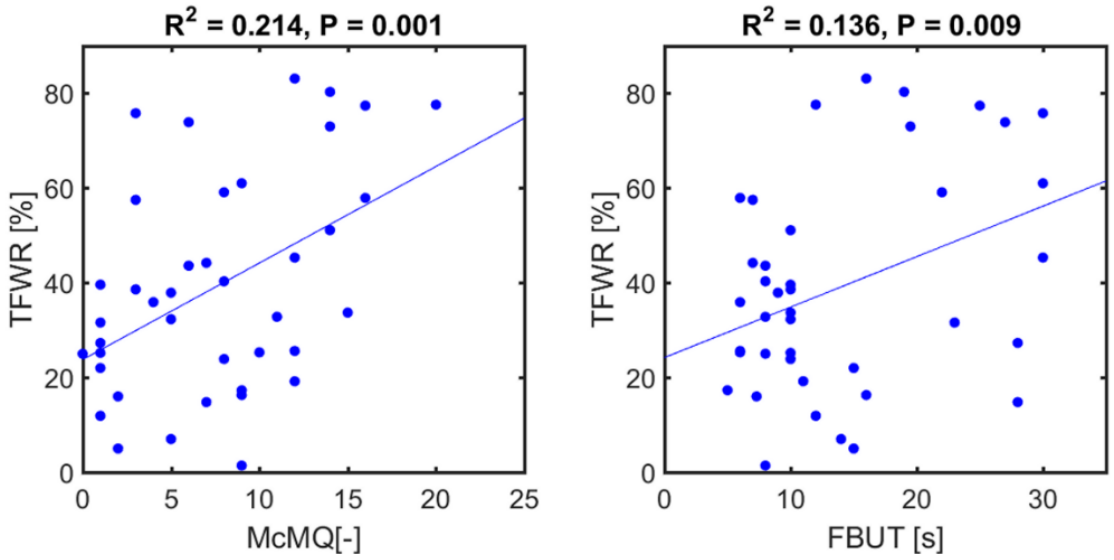


Figure 9. Statistically significant correlations reported in the Experiment 1; TFWR - Tear fluorescein wash-out rate; McMQ- McMonnies Questionnaire score; FBUT - fluorescein tear film break-up time

Low, however statistically significant correlations were reported between TFWR and McMQ ($R^2 = 0.214$, $P = 0.001$) and between TFWR and FBUT ($R^2 = 0.136$, $P = 0.009$).

The summary of the data collected (mean values, standard deviations, ranges) and correlation coefficients between TFWR and other ocular measures acquired in the experiment, including McMQ, FBUT, inferior TMH and blink rate (BR) are displayed below in Table 2.

Table 2. A summary of the data acquired in Experiment 1

Ocular measure	Mean ± SD	Range	Correlation with TFWR
Age [y/o]	32 ± 14	[21, 70]	$R^2 = 0.004$, $P = 0.348$
McMQ [-]	7.6 ± 5.2	[1.0, 20.0]	**$R^2 = 0.214$, $P = 0.001$***
FBUT [s]	14.1 ± 8.0	[5.0, 30.0]	**$R^2 = 0.136$; $P = 0.009$***
TMH [mm]	0.22 ± 0.07	[0.10, 0.40]	$R^2 = 0.015$; $P = 0.225$
BR [min^{-1}]	17 ± 7	[6, 30]	$R^2 = 0.006$; $P = 0.311$
TCR [%/30s]	28 ± 20	[5, 67]	$R^2 = 0.001$; $P = 0.817$
TFWR [%/30s]	39 ± 23	[2, 83]	-

*SD- standard deviation; McMQ – McMonnies Questionnaire score; FBUT – fluorescein tear film break-up time; TMH – tear meniscus height; BR - blinking rate; TFWR – Tear Fluorescein Wash-out Rate, TCR - tear clearance rate; * denotes statistical significance*

E1.4. Repeatability

The repeatability of the proposed TFWR technique was assessed. Ten randomly selected volunteers from the group of subjects were measured several times. All measurements were performed at the same time of the day. Firstly, TFWR was assessed in the same manner, as described in the Methodology section. Subsequently, the eye was carefully rinsed with saline solution to wash out the remaining fluorescent dye and a 10-minute break was

followed before taking another measurement. Following this, a short measurement of the remaining fluorescence intensity was performed for a period of 10 seconds. In all cases no fluorescence was observed in the tear film before recording the subsequent ESP video. A total of 8 measurements of the image intensity decay were performed per subject. There was a statistically significant positive correlation between the mean value of TFWR and its standard deviation ($R^2 = 0.806$, $P < 0.001$) indicating that the repeatability suffers with greater mean TFWR. Repeatability ranged from 2% to 42%, which all indicates that TFWR is highly subject-dependent.

E1.5. Observations

This experiment provided a mode for the clinically applicable TTR observation. Profilometry-based measurements of TFWR can track subtle changes in tear film dynamics. This technique is not time-consuming, it is easy to perform, utilizes low concentration of fluorescent dye and is performed with a commercially available clinical instrument. These characteristics make it more clinically applicable than the standardized technique used for TTR estimation. It can be used to analyse changes occurring in the tear film as a manifestation of the early phase tear film dynamics and, due to high spatial resolution of the device, it is not limited by the effect of corneal permeability to fluorescein. Fluorescein profilometry follows tear dynamics not only on the restricted area of the focal diamond, but on the entire exposed corneo-scleral surface. With the custom-written MATLAB software one can choose the area of analysis. The main limitation is that the tear clearance followed with this method cannot be considered quantitative, until fluorescein is instilled with the strip and thus its volume and concentration cannot be predicted. This should be addressed in the future studies. After improving the blinking mechanism

control and choosing the optimal volume and concentration of the dye to observe the best image and quantify the TFWRs, the repeatability and applicability of this method should visibly increase. Future studies should guarantee standardizing the protocol of measurements. The differences between values of TFWR and those reported in the literature for TTR assessed with fluorophotometry are evident, hence, possible correlations between measures of tear film dynamics assessed with fluorescein profilometry and fluorophotometry require further investigation.

E2. EXPERIMENT 2. OCT-BASED DYNAMIC MENISCOMETRY

As mentioned in ***Chapter I***, geometrical parameters of the tear menisci may depend on many biophysical and external factors and while the environmental impact can be controlled and maintained, the subject-related aspects are difficult to regulate. Methodology for automatic quantitative extraction of tear meniscus parameters is needed and such automatic protocol has been proposed by Bartuzel et al.[200] from the Biomedical Signal Processing Group of Wroclaw University of Science and Technology [1]. This software implements an image-processing algorithm designed for quantitative assessment of tear meniscus parameters based on dynamic image acquisition with OCT. This algorithm performs well for different types of conjunctival sac morphologies, wide range of post blink dynamics and varied size of tear menisci.

A modified version of this software was used to extract the tear meniscus parameters assessed during the longitudinal study of contact lens wearers. Firstly, performance of this method was tested, and the results of this testing were reported in the following sub-chapter. In this experimental study, three different methodologies for inferior tear meniscus parameters evaluation are compared. One, that is based on the K5M *Tear Meniscus Height* built-in software and allows manual measurements of the tear meniscus height based on a

[1] The author would like to acknowledge Maciej Bartuzel, MSc from Biomedical Signal Processing Group of Wroclaw University of Science and Technology in Poland for developing the MATLAB software for tear meniscus morphology assessment with optical coherence tomography, which modified version was used to perform the image analysis in the following experiment.

single infra-red *en face* static image of the subjects' lower tear meniscus. This method is the simplest and, by far, the most commonly applied in clinical setting.

The second method was used to evaluate TMH, TMD and TMA based on a single, static acquisition performed with OCT at a 2-second margin after the blink. This technique corresponds with the one used by Zheng et al. to assess tear meniscus height for TCR analysis. This is the most commonly applied OCT-based type of meniscometry. Static tear meniscus height (S-TMH), static tear meniscus depth (S-TMD) and static tear meniscus area (S-TMA) were acquired with this technique.

Lastly, the third method utilizes the abovementioned software to perform dynamic meniscometry. Dynamic tear meniscus height (D-TMH), dynamic tear meniscus depth (D-TMD) and dynamic tear meniscus area (D-TMA) will be assessed on the basis of dynamic sequence of B-scans recorded after the blink with spectral-domain OCT. Bartuzel indicates that any observable changes in the early post-blink meniscus parameters are most likely related to the longitudinal movement of the eye[219] rather than to the tear meniscus formation occurring immediately after each blink, that corresponds to the phase of tear film build-up[220,221]. Therefore, a single static acquisition-based assessment of tear meniscus parameters acquired at a random moment after the blink should be viewed with caution. Tear meniscus undergoes dynamic changes after each blink and thus the dynamic meniscometry should provide a more reliable and in-depth estimate of the geometrical parameters of tear meniscus, including TMH, TMD and TMA.

E2.1. Methodology

The study recruited fifty-five healthy, young subjects (39F and 17M), aged (mean ± standard deviation) 26 ± 4 y/o, ranging from 20 to 37 y/o. The exclusion criteria and protocol of measurements was consistent with the Baseline visit of ***the longitudinal study of biomarkers' trends*** (***Chapter III***). Figure 10 shows an exemplary B-scan of the lower central eyelid margin and the inferior tear meniscus; it was acquired for one of the subjects with spectral-domain OCT (Copernicus, Optopol, Poland). Additionally, the exemplary tear meniscus measures, which can be assessed with the custom-written MATLAB software, are encircled and enlarged in Figure 10.

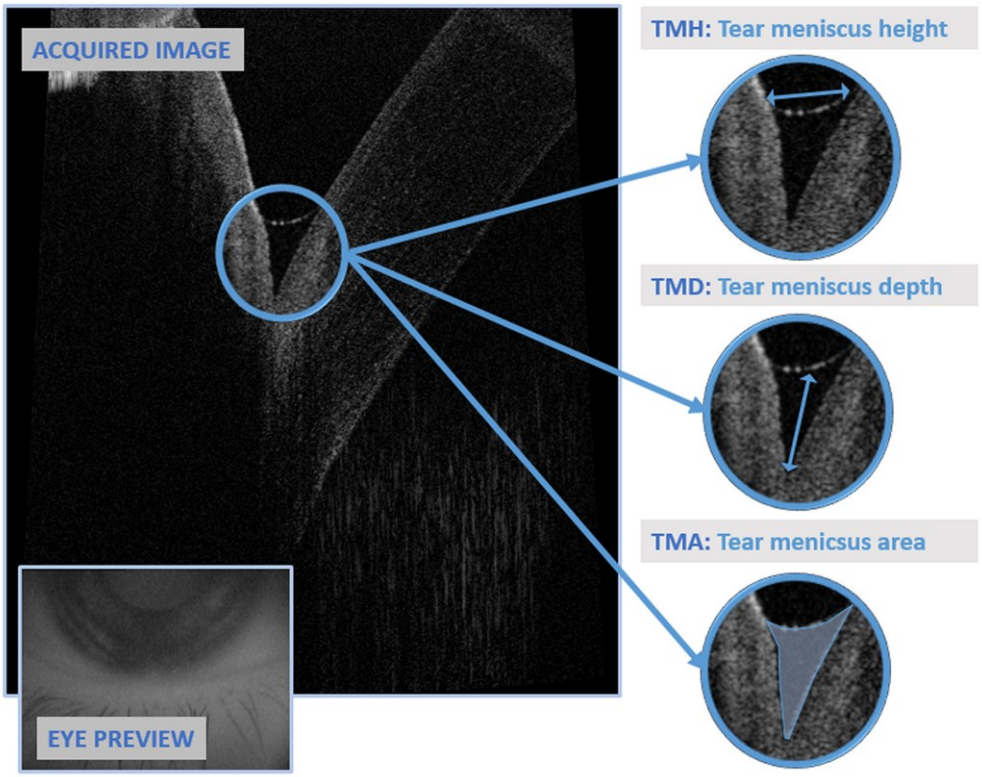


Figure 10. Exemplary B-scan of the inferior tear meniscus obtained for one of the subjects with OCT and measurements that can be performed automatically with the proposed MATLAB software

E2.1.1. OCT-based dynamic meniscometry

Additional lens was attached to the device to visualise the anterior segment of the eye. The *Animation mode* of the device was selected to acquire images of the central inferior tear meniscus in a form of multiple B-scans. The scanning angle and width were set to 90° and 4 mm, respectively. The maximum possible number of B-scans (which is 90) was chosen in this setting. The B-scan plane, with a maximum of 1800 A-scans was central (with respect to the iris outline) to the posterior region of the eye-eyelid junction and normal to the eyelid. The maximum possible number of 90 B-scans allowed in that setting was chosen. The subjects were asked to look straight ahead in the mirror, which simulates distant gaze, and to blink freely until advised, while refraining from head and eye movements. To make sure that the sequence captures the blink period right after the B-scans sequence was initialised, the subjects were asked to blink once and to refrain from blinking. Each sequence comprised of 90 frames, which corresponded to total recording time of 3.75 seconds and could be easily divided into three intervals. After each blink, the tear meniscus parameters stabilise, thereby it is possible to distinguish frames that correspond to the *post-blink phase*, when the longitudinal movements of the eye play a major role, and those which correspond to the *interblink phase*, when the tear meniscus parameters are slightly fluctuating. The mean values of the inferior TMH, TMD and TMA from all the frames constituting the interblink interval are automatically assessed and calculated with the custom-written software. Figure 11 shows values of TMH calculated with the software from B-scan sequence. When using the proposed algorithm, one can avoid the post-blink tear meniscus non-confluence, which affects the estimation, resulting in more precise estimate of tear meniscus geometrical parameters.

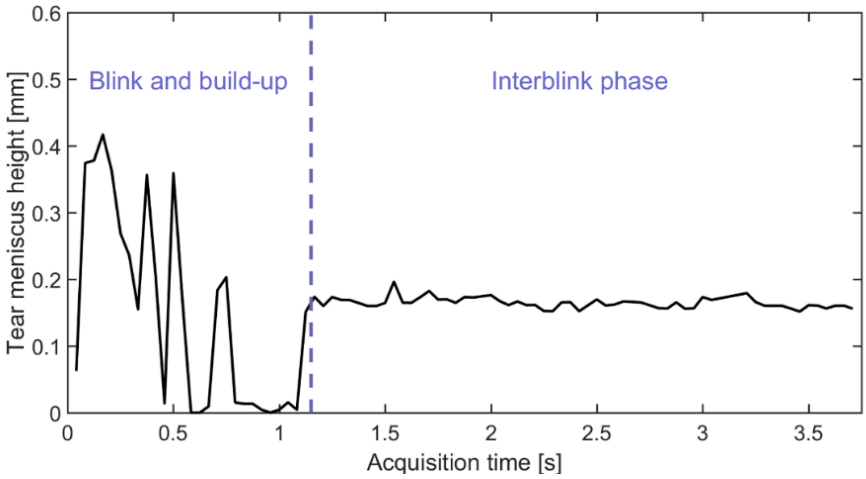


Figure 11. An exemplary result of TMH dynamics calculation post-blink. Dashed grey line indicates an arbitrary division of the TMH temporal changes into a blink and post-blink phase

The use of the custom-written software allows more control over the tear meniscus parameters following each blink and to delete the outlying values from the final estimation. This approach considers small movements of the eye, longitudinal movements of the globe after each blink and continuous changes of tear menisci, which cannot be observed based on a single, static B-scan acquired at a random moment after the blink. The inferior TMH is not constant and increases with time after the blink[222]. Right after the blink, the rate of change is very rapid, resulting in a relatively stable TMH after a short time and small standard deviation, which could be observed with the proposed algorithm. Additionally, this software provides the time of tear meniscus build-up after the blink. The algorithm described above was used to acquire D-TMH, D-TMD and D-TMA, which are the dynamic analogues of static measures of tear meniscus (S-TMH, S-TMD and S-TMA), described in the subsection below.

E2.1.2. OCT-based static meniscometry

In this method, one B-scan from the sequence acquired with OCT was chosen. This B-scan corresponds to the tear meniscus after approximately 2 seconds post-blink. Tear meniscus parameters (S-TMH, S-TMD and S-TMA) were measured manually with use of *ImageJ* software (US National Institutes of Health, Bethesda, MD).

To perform the measurements, images needed to be resized, as OCT software compensates for optical aberrations when displaying the images on the screen (Figure 12). After this procedure, if the configuration of scanning angle of 90° and scan width of 4 mm was set, then each pixel of the acquired image corresponds to approximately 4 µm.

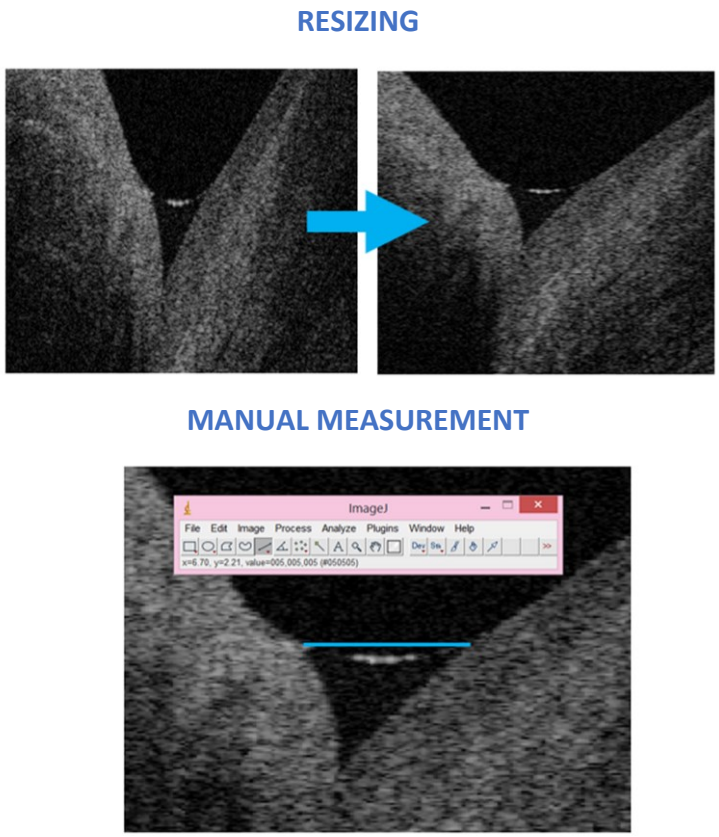


Figure 12. Two main steps to perform when analysing the OCT B-scan with ImageJ; Step 1 - resizing the image in MATLAB with 'resize' function; Step -2 measuring tear meniscus parameters

E2.1.3. K5M-based tear meniscus height

K5M allows measurements of the TMH based on a single infra-red *en face* image of the inferior tear meniscus. Lower central TMH of each subject was recorded with the use of *TF-Scan* function of K5M called *Tear Meniscus Height.* Default settings were used. To assess TMH of both eyes of each subject, subjects were asked to fixate on the central target. They could blink naturally during the entire procedure. The tear meniscus images were captured at around two seconds after the subject's natural blink. K5M allows measurements of TMH in infra-red spectrum, which is invisible to human subjects. This minimizes the effect of reflex tearing caused by excessive illumination. The infra-red illumination mode was switched-on, and measurements were performed in a dimly lit room to increase contrast of the images by limiting background illumination. The camera was positioned on the central lower eyelid of the subject and the reflected rings were brought into focus.

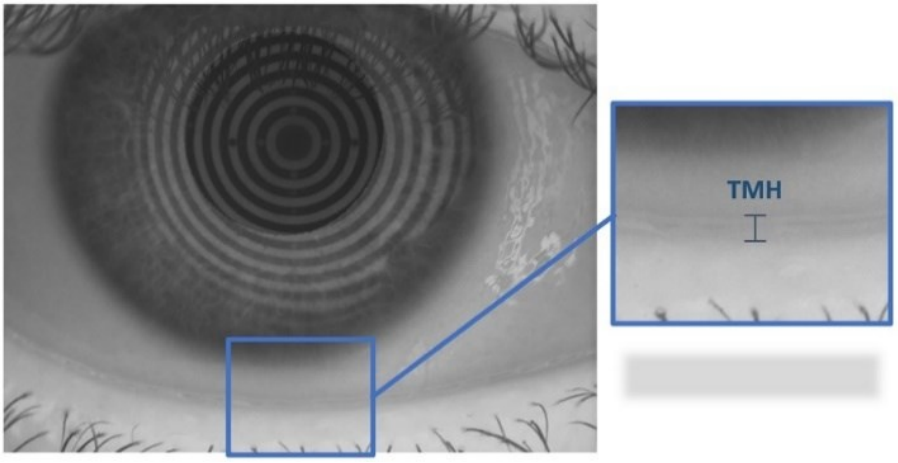


Figure 13. An exemplary image of the inferior tear meniscus height acquired with K5M

After the image is acquired, the K5M automatically opens the image analysis window and enables manual TMH measurements with the use of built-in callipers. The length of a line (in millimetres) along the chosen dimension is displayed when clicking on both parallel edges of the central tear meniscus. Single assessment was performed for each eye and the images were stored automatically. Figure 13 presents an exemplary image of the eye, acquired for one of the subjects with Oculus K5M *Tear Meniscus Height* imaging tool. A close-up of the tear meniscus section, on which the measurements were performed, is also displayed.

E2.2. Results

Table 3. Tear meniscus height measures assessed with different methods

OCT-based static method			
Ocular measure	TMH [mm]	TMD [mm]	TMA [mm^2]
Mean ± SD	0.21 ± 0.05	0.18 ± 0.06	0.015 ± 0.010
Median	0.21	0.18	0.025
Range	[0.09, 0.30]	[0.08, 0.36]	[0.051, 0.070]
OCT-based dynamic method			
Mean ± SD	0.27 ± 0.12	0.16 ± 0.02	0.012 ± 0.005
Median	0.26	0.16	0.012
Range	[0.05, 0.54]	[0.11, 0.20]	[0.004, 0.022]
K5M-based method			
Mean ± SD	0.24 ± 0.06	-	-
Median	0.24	-	-
Range	[0.15, 0.46]	-	-

SD - standard deviation; TMH - tear meniscus height; TMD - tear meniscus depth; TMA - tear meniscus area

Table 3 shows the mean and median values, ranges and standard deviations of the tear meniscus geometrical measures reported in the experiment. Additionally, the statistical agreement between the dynamic and static OCT-based method of tear meniscus evaluation

was further inspected. Figure 14 shows a scatter plot with the line of equality between left eye TMH assessed during the baseline visit with static OCT-based meniscometry (x-axis) and dynamic OCT-based meniscometry (y-axis). Figure 15 represents the Bland-Altman plot of these two OCT-based meniscometry methods.

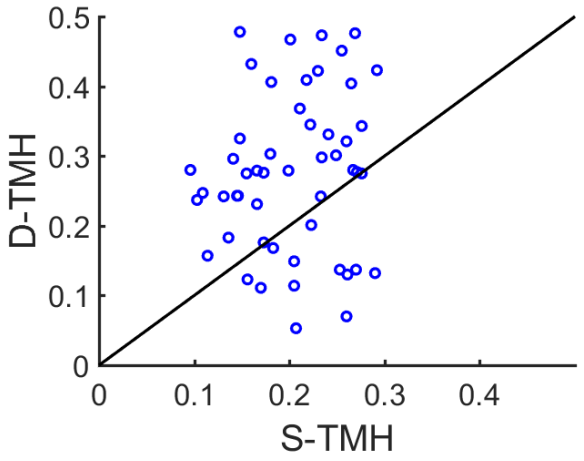


Figure 14. Scatter plot with line of equality between S-static and D-dynamic OCT-based left eye central inferior tear meniscus height (TMH)

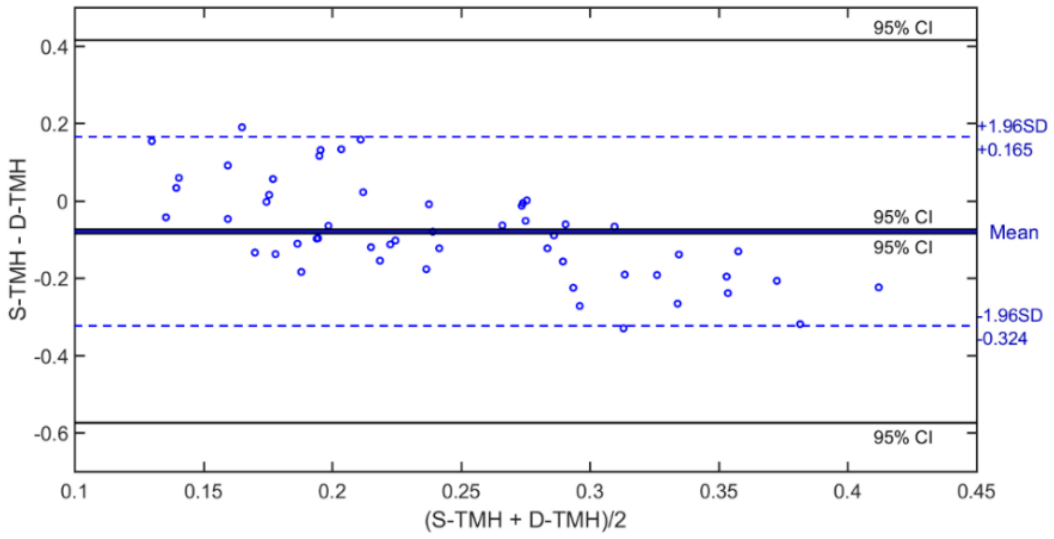


Figure 15. Bland-Altman plot (difference plot - average of two methods against the difference) comparing the two OCT-based measurements of tear meniscus height; S-TMH - static method based on a single scan; D-TMH - dynamic method based on 90 B-scans; CI - confidence interval; SD - standard deviation

Table 4 shows the linear correlation coefficients between different measures of tear meniscus geometrical parameters assessed during this experiment and their corresponding *P*-values.

Table 4. The linear correlation coefficients and corresponding P-values between different geometrical measures of tear meniscus morphology

Meniscus measure	**S-TMH**	**S-TMD**	**S-TMA**	**D-TMH**	**D-TMD**	**D-TMA**
TMH_{K5M}	*R* = 0.813 *P* = 0.324	*R* = -0.001 *P* = 0.626	*R* = 0.016 *P* = 0.477	*R* = 0.176 *P* = 0.127	*R* = -0.049 *P* = 0.978	*R* = -0.123 *P* = 0.693
S-TMH	-	***R* = 0.893** ***P* < 0.001***	***R* = 0.879** ***P* < 0.001***	*R* = -0.035 *P* = 0.693	***R* = 0.400** ***P* <0.001***	*R* = 0.100 *P* = 0.254
S-TMD	-	-	***R* = 0.937** ***P* < 0.001***	*R* = -0.051 *P* = 0.561	***R* = 0.424** ***P* < 0.001***	*R* = 0.211 *P* = 0.015
S-TMA	-	-	-	*R* = -0.061 *P* = 0.491	*R* = 0.402 *P* < 0.001*	*R* = 0.165 *P* = 0.058
D-TMH	-	-	-	-	***R* = -0.465** ***P* < 0.001***	***R* = -0.617** ***P* < 0.001***
D-TMD	-	-	-	-	-	***R* = 0.707** ***P* < 0.001***

S - static, D-dynamic; TMH -tear meniscus height; TMD - tear meniscus depth; TMA - tear meniscus area; TMH_{K5M} - KM5-based tear meniscus height

E2.3. Observations

Considering the combination of interfering factors related to head, eye and eyelid movements, static acquisition of tear meniscus with OCT may not provide reliable estimates of tear meniscus geometrical parameters. The clinical utility of dynamic OCT-based tear meniscus measurement lies in the necessity of continuous acquisition of post-blink tear meniscus geometry. This is to provide precise estimate of its parameters such as height, depth, and area of the cross-section calculated as mean values over a few seconds

post blink. Statically significant linear correlation was not noted between OCT-based and K5M-based tear meniscus height, which may suggest that these two methods give different insight into tear meniscus parameters. OCT-based method allows more in-depth visualization of tear menisci. Apart from that, the tear meniscus measures assessed with OCT-based method correlate with each other.

When analysing the scatter plot, one can see that these two methods of OCT-based assessment - the dynamic and the static one - are not entirely in agreement, with dynamic meniscometry-based values tending to be higher. Most values in the Bland-Altman graph are within the 95% confidence interval. Visible linear trend was observed in the Bland-Altman graph, which may suggest that the two methods of tear meniscus assessment are not in agreement. Based on the analysis presented above one can conclude that the different methods of tear meniscus evaluation provide different insight into ocular physiology and different tear meniscus geometrical parameters.

E3. EXPERIMENT 3. OCT-BASED ASSESSMENT OF EARLY PHASE TEAR CLEARANCE

In 2014 Zheng et al.[169] attempted to exploit the effect of Krehbiel flow to study an early-phase tear clearance by means of OCT. This technique was used to study the decrease in TCR as a function of age[169] and its performance was compared with the Schirmer-based FCT. The abovementioned procedure is based on changes of tear meniscus parameters after application of 5 μL of 0.9% buffered saline solution. Temporal changes of TMH and TMA over time were followed[169]. The OCT-based method of TCR evaluation is less invasive and relatively shorter in comparison with other methods of tear clearance assessment. Additionally, it does not require topically applied fluorescein, which is poorly accessible in some European countries and utilizes the device which is more commonly used in clinical setting than fluorophotometry or scintigraphy. In contrast to FCTs, OCT-based method does not only define tear flow in a form of a single value, but also allows tracking temporal changes in tear fluid characteristics objectively and qualitatively.

In the study of Zheng et al. the OCT-based TCR was correlated positively with FCT scores and negatively correlated with the distance between the lacrimal punctum and Marx's line and with ocular conditions known to interrupt tear flow and often accompanied by DED symptoms, like the degree of conjunctivochalasis and the degree of lacrimal punctum protrusion. In the following experiment, further development in the OCT-based assessment of TCR[169,171] was made. Since 2014, when the technique was proposed by Zheng, a second study was conducted by the same team, utilizing Polymethylmethacrylate particles to study the phenomenon of Krehbiel flow. In the study of Zheng et al. The temporal changes in tear meniscus morphology, including TMH and TMA were assessed based on single, static

acquisition, after 30 seconds, 1 minute and every consecutive minute up to 5 minutes post instillation. Dynamic changes in these parameters were followed to calculate TCR. Measurements were repeated 3 times in intervals of at least 15 minutes between them.

The experimental method for TCR assessment proposed here is based on the observation of the tear meniscus morphology changes post-saline instillation with a spectral domain OCT. What differentiates this method from the one proposed by Zheng et al., is the application of the dynamic meniscometry algorithm proposed in ***Experiment 2*** for tear meniscus estimation. The dynamic acquisition comprising of 90 B-scans was performed for each meniscometry. Automatic estimation of tear meniscus parameters post-blink, simplifies the procedure and enhances the precision of the measurement by allowing an objective assessment[200]. An additional aim was to compare the TCR estimates with the tear film measures commonly used in a clinical setting for DED diagnosis. This experiment presents the first stage of testing of the OCT-based TCR and shows its potential to become a clinical diagnostic test for DED.

E3.1. Study protocol

Fifty-five healthy, young subjects (36F and 19M) aged (mean ± standard deviation) 26 ± 4 y/o ranging from 20 to 37 y/o volunteered for this study. The group comprised of the subjects taking part in ***the longitudinal study of biomarkers' trends (*****Chapter III**) and the data were collected in parallel with the baseline, preliminary visit of this study, thus the protocol of measurements and inclusion/exclusion criteria were the same. The evaluation sheet for the Baseline visit was attached at the end of this dissertation (see ***Appendix 5***). Summarizing, the protocol of measurement consisted of:

- OSDI and DEQ-5 questionnaires (see ***Appendix 2);***
- the review of medical history (see ***Appendix 3***);
- TMH measurement with K5M (as shown in the Figure 13 and described in the ***Experiment 2***);
- tear osmolarity measurements acquired from the inferior temporal tear meniscus with TearLab Osmolarity System (Tear Lab Corp., San Diego, CA, US);
- Non-invasive Keratograph® Tear Film Break-up Time (NIKBUT) automatic measurements with K5M;
- slit lamp biomicroscope anterior eye examination protocol (see ***Appendix 4***).
- The TCR estimation based on dynamic inferior tear meniscus morphology acquired with spectral domain OCT (Copernicus, Optopol Ltd., Poland) as proposed in the previous experiment. After a short break, TCR assessment was followed by:
- fluorescein tear film break-up time (FBUT) estimation;
- LWE scoring with lissamine green and infrared meibography recording with K5M.

E3.2. Methodology

Dedicated lens system was attached to the OCT probe to visualize the anterior segment of the eye. Spectral domain OCT (SOCT Copernicus, Optopol Ltd., Poland) was used to acquire B-scans of subject's lower central eyelid morphology and inferior tear meniscus. Measurements were performed in mesopic conditions (Figure 16) to increase contrast of B-scans. To minimize the effect of fluid viscosity and density on the tear film and tear meniscus retention times, saline solution was used. Following the evidence that TCR and TMH could vary with the time of the day[65,124] measurements were performed in the morning and not more than 4 hours since the subjects awakening time.

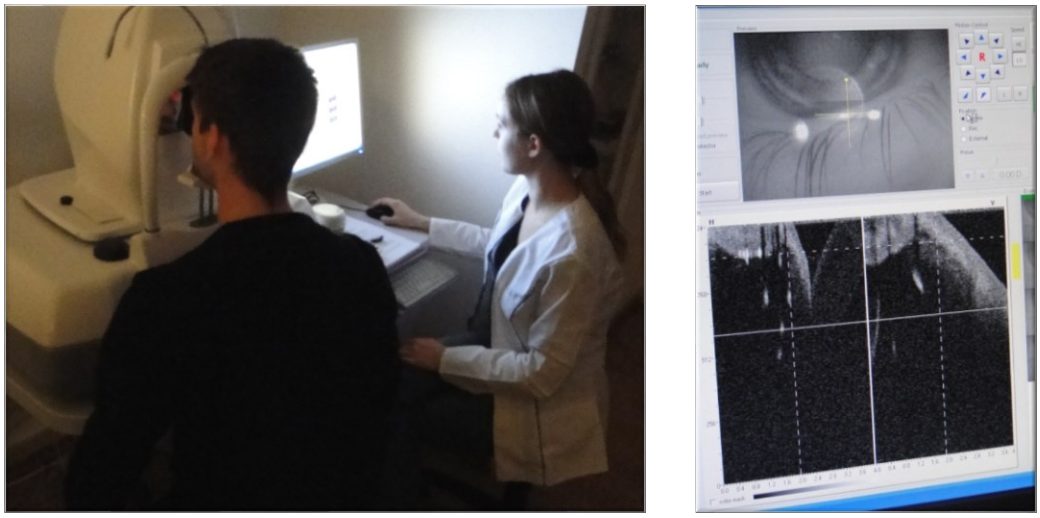


Figure 16. OCT-based TCR assessment; Left: measuring station with the subject in position; Right: OCT built-in software's interface

Additionally, changes in tear volume and TCR with age were noted in the previous studies[169,204], so the age distribution of the participants for this experiment was kept as narrow as possible in order to prevent their age from affecting the results. Temperature and relative humidity in the laboratory were stable and monitored with a thermo-hygrometry device (C3121, Comet, Czech Republic) because tear meniscus parameters vary highly under the influence of environmental conditions.

Each subject was instructed to sit comfortably, place the chin on the chinrest and press the forehead against the bar of the instrument and do not move away from the device for the whole duration of the procedure, unless clearly instructed to do so. Subjects were instructed to look at the reflection of their eye in a dedicated mirror, which simulates a primary gaze position.

Based on the procedure presented by Zheng *et al.*[169], spectral domain OCT was used to record the dynamic changes of the inferior tear meniscus. Firstly, three consecutive

dynamic acquisitions of the central inferior tear meniscus image of each subject were performed. The baseline tear meniscus morphology measurements were averaged. Baseline values of the OCT-based measurements of D-TMH, D-TMD and D-TMA were used in ***the longitudinal study of biomarkers' trends*** as a separate ocular measure and potential biomarker (***Chapter III***). The basal tear meniscus morphology will be later referred to as the *Baseline* (BL) value. Subsequently, subjects were instructed to tilt their head backwards, look up and away from the practitioner and, subsequently, the application of 5 μL of room-temperature 0.9% buffered saline solution was performed with an automatic micropipette (Topscien S, Biosens Ltd, Poland) into the subject's conjunctival sac. All measurements were performed on the left eye of each subject to facilitate fluid instillation by a right-handed practitioner. The method of application was performed close to the tarsal side of the left eye of each subject and was not associated with any observable or reported discomfort.

Right after the instillation the subjects had to return to their originally adopted position. This was to ensure quick image acquisition after instillation. If necessary, small adjustment of the probe position were performed and consecutive B-scans sequence was acquired. This B-scan sequence will be later referred to as a '0' minute acquisition. After this acquisition, stopwatch was turned on and the 3-minute period of measurements was initiated. Consecutive measurements were performed at 30 seconds, one minute, two and three minutes post-instillation to follow tear parameters decay with time. Subjects could blink naturally during the entire time and they only had to refrain from blinking for 90-B-scan sequences to be acquired. The 3-minute period of observation was adapted, as this amount of time was proven to be sufficient for the tear meniscus of young, healthy subject to come back to its basal form after instillation. one-minute break between

measurements was kept, allowing to save the sequence in the OCT's database and to adjust the OCT probe position if necessary. As mentioned before, the animation sequence contains 90 B-scans, thus the time the subjects must keep their eyes open is less than 3.75 seconds.

The aim of this 3-minute assessment was to follow the dynamic decay of TMH, TMD and TMA post instillation with time. The $TCR_{TMH,}$ TCR_{TMD} and TCR_{TMA} were estimated as a percentage decrease in D-TMH, D-TMD or D-TMA at 30-sceond margin post-instillation, as proposed by Zheng et al. Each B-scan sequence was analysed with the use of the custom-written MATLAB software described in the ***Experiment 2***.

E3.3. Reproducibility

To test the reproducibility of OCT-based measurements of TCR, multiple measurements of TMH post-instillation dynamics have been performed and TCR calculated for one, randomly chosen subject at each day of seven days period. This subject was not a contact lens wearer and was required not to use any topically applied substances at least the day before and for the six-day period of measurements. Each measurement was performed at the same time of the day and the time of measurement was set to 5 minutes post instillation. Figure 17 shows all curves of dynamic changes in tear meniscus height acquired for that subject. The coefficient of variation of the OCT-based TCR_{TMH} estimates was reported to be around 14.9%. The mean reported TCR_{TMH} throughout the time-course of one week for this subject was estimated to be 24 ± 4% at a 30-second margin post-instillation.

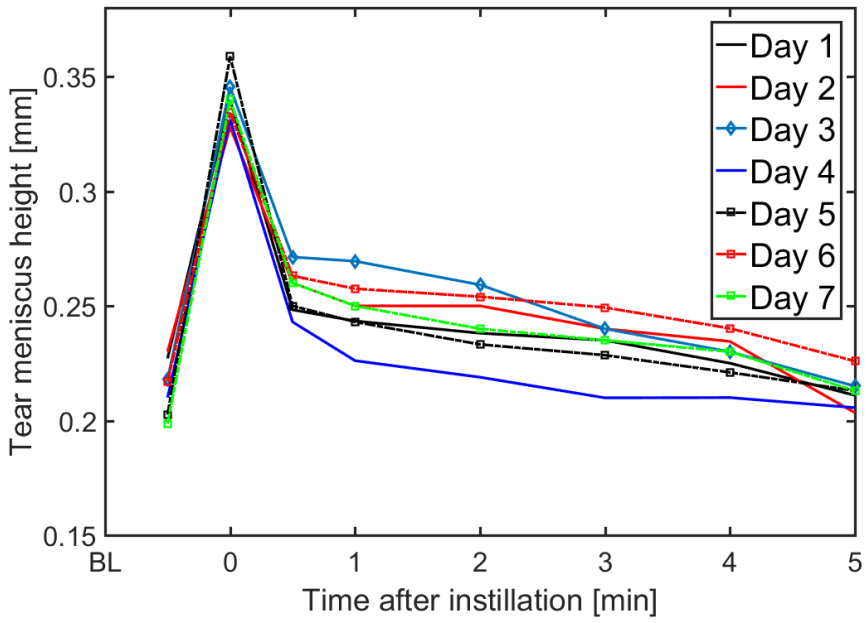


Figure 17. The dynamic changes of tear meniscus height reported for one of the subjects measured once per day in the morning for a period of one week.

E3.4. Results

Mean temperature and mean relative humidity in the laboratory were estimated to be (mean ± standard deviation) 24.5 ± 1.2 °C and 32.2 ± 4.7 %RH, respectively. The group's mean and median values, standard deviations and ranges of the measures reported in the experimental study are shown in Table 5.

Table 5. A summary of tear clearance rates acquired in the Experiment 3

Ocular measure	**TCR_{TMH} [%/30s]**	**TCR_{TMD} [%/30s]**	**TCR_{TMA} [%/30s]**
Mean	22 ± 21	18 ± 19	29 ± 31
Median	21	18	31
Range	[-14, 74]	[-29, 69]	[-88, 95]

TCR - Tear clearance rate based on: TMH - tear meniscus height, TMD - tear meniscus depth and TMA - tear meniscus area

The TCRs were tested for normality with Lilliefors test and the null hypothesis was not rejected for TCR_{TMH} (P = 0.129), TCR_{TMD} (P = 0.479) nor TCR_{TMA} (P = 0.144). Estimated time required for tear meniscus to come back to its basal level after the instillation of 5 µL of saline solution was estimated to be around two minutes in young, healthy subjects, which agrees with the study of Zheng *et al.* The reduction of TMH, TMD and TMA at 30 seconds post-instillation was significant. Figure 18, Figure 19 and Figure 20 show the group mean of TMH, TMD and TMA dynamics, respectively. These dynamic changes were used to calculate the corresponding TCRs.

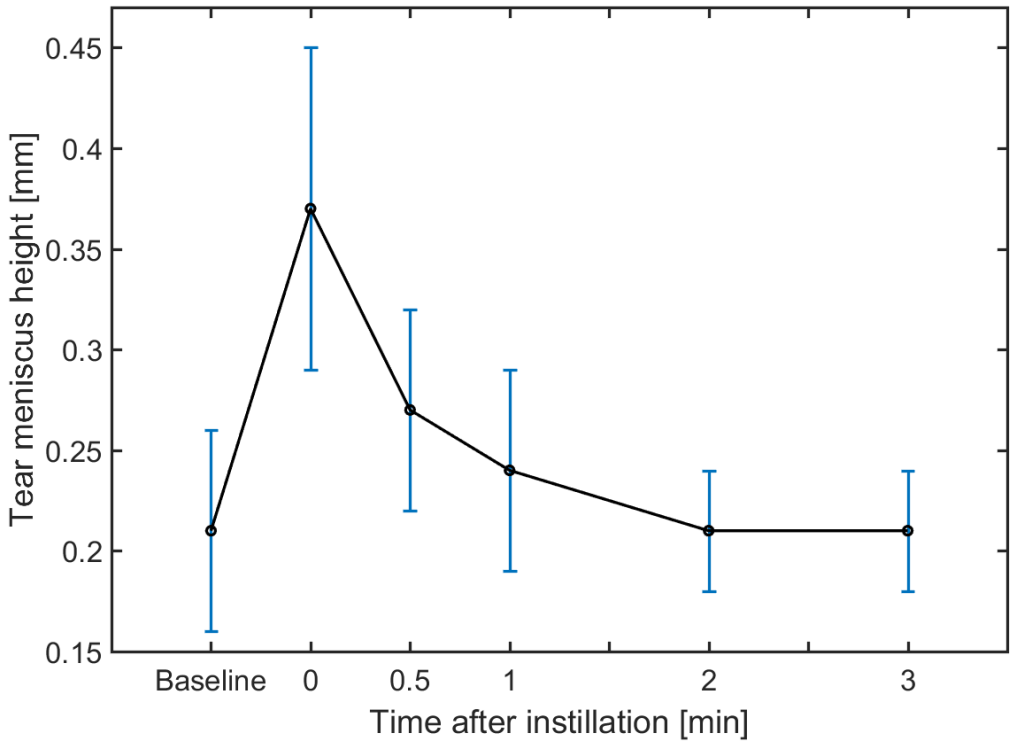


Figure 18. The group mean TMH dynamics post saline instillation. Error bars indicate ± one standard deviation

The baseline value of TMH was estimated to be (mean ± standard deviation) 0.21 ± 0.05 mm. Right after the instillation, the increase in TMH was statistically

significant ($P < 0.001$) and TMH was estimated to be around 0.37 ± 0.08 mm. 30 seconds after instillation TMH decreased significantly ($P < 0.001$) and was on average (mean ± standard deviation) 0.27 ± 0.05 mm. TMH also changed significantly between 30 seconds and one-minute post instillation ($P < 0.001$), decreasing to 0.24 ± 0.05 mm and between one and two minutes ($P < 0.01$), decreasing to 0.21 ± 0.03 mm. TMH change between second and third minute post-instillation was not statistically significant ($P = 0.357$).

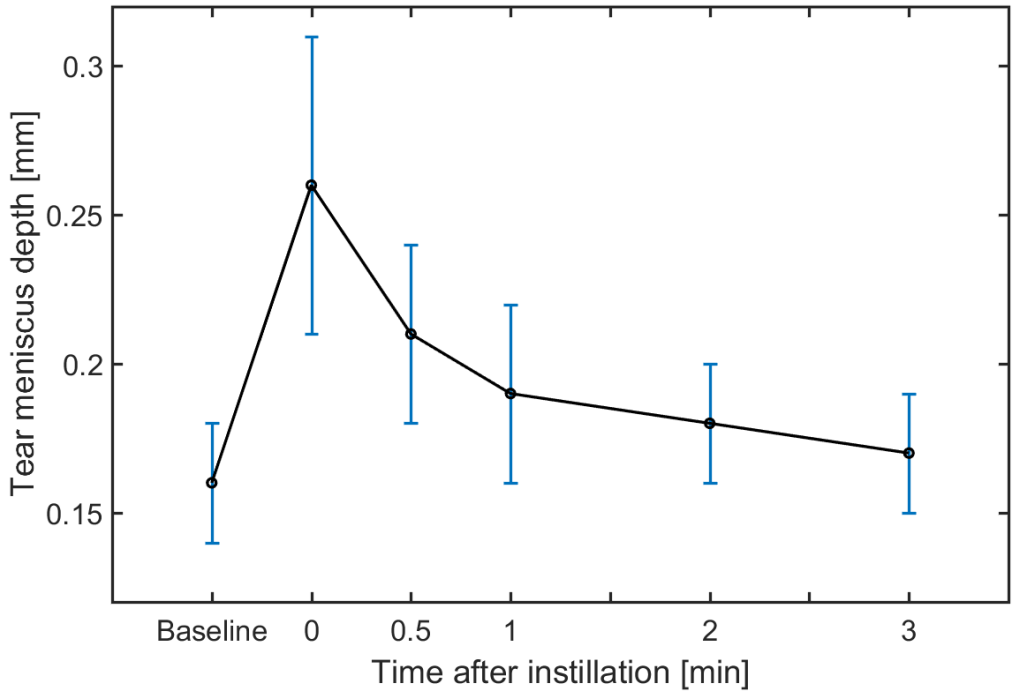


Figure 19. The group mean TMD dynamics post saline instillation.

Error bars indicate ± one standard deviation

The baseline value of TMD was estimated to be (mean ± standard deviation) 0.16 ± 0.02 mm. The increase of TMD measured immediately after instillation was statistically significant ($P < 0.001$) and TMD was estimated to be around 0.26 ± 0.05 mm. Following 30 seconds of natural blinking, TMD decreased significantly ($P < 0.001$) and was on average 0.21 ± 0.03 mm. TMD also changed significantly between 30 seconds and

one-minute (P = 0.032), decreasing to 0.19 ± 0.03 mm and between one and two minutes (P = 0.005), decreasing to 0.18 ± 0.03 mm and reaching its basal (pre-instillation) level. Change in TMD between second and third minute post-instillation was not statistically significant (P = 0.264).

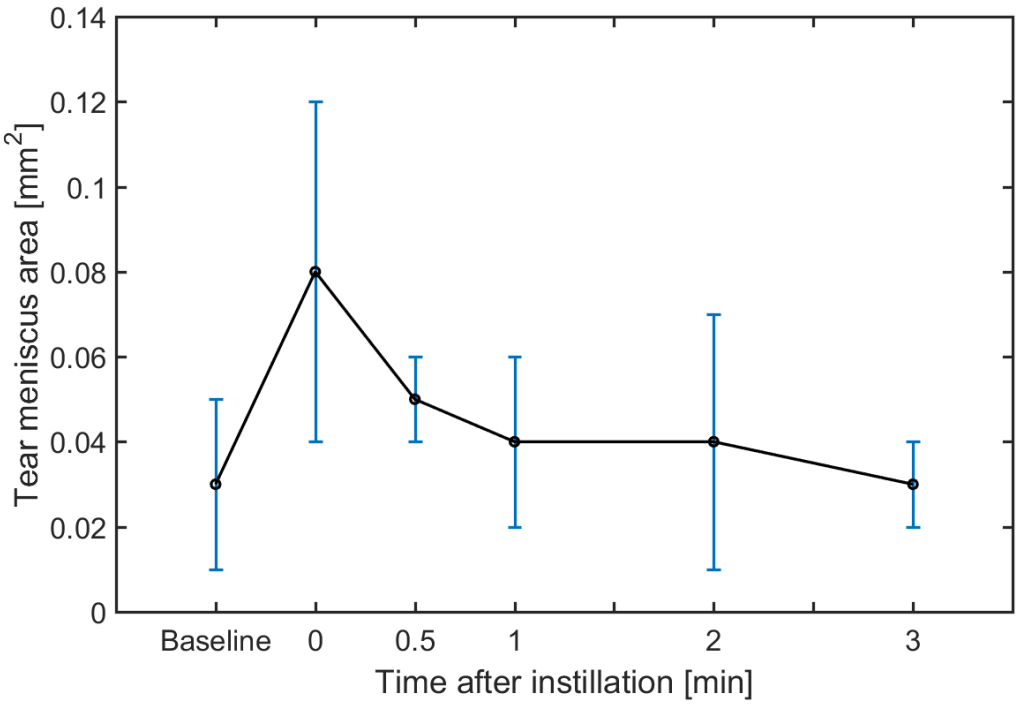


Figure 20. The group mean TMA dynamics post saline instillation.

Error bars indicate ± one standard deviation

The baseline value of TMA was estimated to be (mean ± standard deviation) 0.03 ± 0.02 mm^2. After instillation TMA increased significantly (P < 0.001) to 0.08 ± 0.04 mm^2. Following 30 seconds of natural blinking, TMA decreased significantly (P < 0.001) and was on average 0.05 ± 0.01 mm^2. TMA also changed significantly between 30 seconds and one minute (P = 0.008), decreasing to (mean ± standard deviation) 0.04 ± 0.02 mm^2 and between one and two minutes (P = 0.009) after instillation. Change in TMA between second and third minute post-instillation was not statistically

significant (P = 0.357). Statistically significant linear correlations were evaluated between different measures of TCR (see Table 6).

Table 6. The linear correlation coefficients and corresponding P-values between different measures of OCT-based TCR

Ocular measure	**TCR_{TMD}**	**TCR_{TMA}**
TCR_{TMH}	***R* = 0.843** ***P* < 0.001***	***R* = 0.843** ***P* < 0.001***
TCR_{TMD}	**-**	***R* = 0.872** ***P* < 0.001***

*TCR - tear clearance rate base on: TMH - tear meniscus height, TMD - tear meniscus depth and TMA - tear meniscus area; * denotes statistical significance*

E3.5. Correlation with Fluorescein Wash-out Rate

To test whether two methods of tear clearance estimation: the profilometry-based (***Experiment 1***) and OCT-based (***Experiment 3***) correlate with each other, 30 subjects underwent both procedures. Subjects aged (mean ± standard deviation) 26 ± 5 y/o ranging from 22 to 45 y/o participated in this part of the study. The TMH-based TCR for this group of subjects was estimated as (mean ± standard deviation) 29.7% ± 21.4%, ranging from 5% to 67% at 30-second margin and TWFR as (mean ± standard deviation) 32.5% ± 19.7%, ranging from 2% to 61% at 30-second margin. Figure 21 shows correlation between two methods of tear dynamic assessment presented in ***Chapter II***. Statistically significant linear correlation was not observed between these ocular measures of tear dynamics (R = 0.022, P = 0.433).

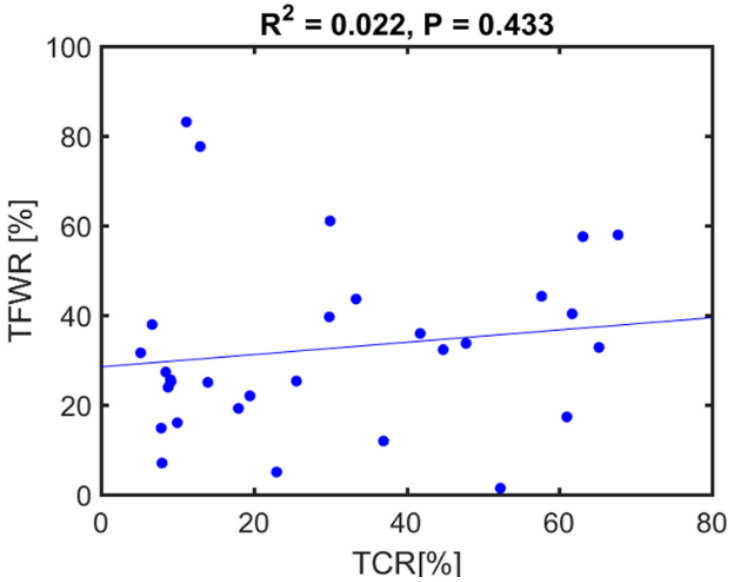


Figure 21. Correlation between profilometry-based assessment (TFWR) and OCT meniscometry-based (TCR) assessment of tear dynamics temporal rates

E3.6. Observations

Generally, findings of Zheng *et al.* and the results of the ***Experiment 3*** suggest that geometrical parameters of the inferior tear meniscus decrease most significantly early after instillation. Zheng suggested possible impact of Krehbiel flow on OCT-based TCR measurements and showed that TCR may rather be a manifestation of an early-phase tear turnover rather than the basal, slow tear turnover assessed with fluorophotometry[169,171].

Experiment 3 further contributes to these developments. TCR reported in the experiment was characterized by large variation among subjects and by relatively good reproducibility. The software helped to enhance precision of tear meniscus morphology measurements with OCT and to monitor and minimize the effect of tear meniscus nonconfluence after each blink on reported estimations[200]. OCT can be used as a rapid, qualitative and quantitative method of determining TCR. With the new algorithm developed, tear meniscus parameters can be calculated considering the nonconfluence of tear meniscus morphology after each

blink, that is, most presumably, connected to the longitudinal eye movements. Traditional clearance tests are either invasive or laborious, and the use of fluorescein has its limitation, because the clearance of tears is not directly measured. Tear clearance measured with OCT is non-invasive and relatively more rapid and simpler to perform than traditionally used tear exchange tests.

It was estimated that for healthy, young subjects the tear meniscus height comes back to its basal state after approximately two to three minutes from fluid instillation. This technique can be additionally used in testing artificial tears retention times[214,215]. TCR was shown to be lower in elderly subjects and symptomatic subjects. The presented correlations between measures assessed in this experiment and TCR can lead to better understanding of the complexity of tear film dynamics and the role of tear clearance in the pathogenesis of DED. Some guidance on this topic has been provided in ***Chapter IV*** in a form of comprehensive discussion of all the parameters reported in this dissertation.

SUMMARY

Measures of tear exchange obtained as results of the aforementioned experimental studies were shown in Table 7, together with the results obtained by Zheng et al.[169] and the mean value of the fluorophotometry-based TTR reported based on comprehensive meta-analysis[121]. TCR assessed with both OCT and fluorescein profilometry is presumed to be the manifestation of an early-phase tear turnover. The rates of profilometry-based TFWR are much higher that the OCT-based TCRs. They are also much more subject-dependent and hence - more variable.

Table 7. A summary of tear turnover measures obtained as the result of the experimental studies presented in this thesis compared with values reported in literature.

Year	Report	Type (number of subjects)	Method	Reported TTR [%]
2009	Tomlinson et al.[121]	Normal (187)	Fluorophotometry	16.2 ± 5.1
2014	Zheng et al.[169]	Young group (30) Elderly group (30)	OCT- TMH-based; OCT -TMA-based; OCT- TMH-based; OCT -TMA-based	35.2 ± 11.0 28.1 ± 12.4 12.4 ± 7.3 6.2 ± 9.1
2017	***Experiment 1***	Normal (40)	Fluorescein; Profilometry	39 ± 23
2017	***Experiment 3***	Normal (56)	OCT- TMH-based; OCT- TMD-based; OCT - TMA-based	21 ± 20 18 ± 18 28 ± 30

TMH – Tear meniscus height; TMA – tear meniscus cross-section area

Summarizing, the fluorophotometry-based evaluation of TTR, its limitations and TTR's impact on the ocular surface health were widely studied, however TTR measurements were

not performed in the clinical setting due to the complexity of the applied methodology and the need for specialized equipment. Proposed simpler alternatives - FCTs are either invasive or based on subjective assessment and fail to follow dynamic changes naturally occurring in the tear film.

Two experimental studies were proposed to investigate tear turnover in a more clinical, simpler manner which allows tracking dynamic changes occurring in the tear film or tear meniscus. Further investigation is needed to standardize the procedures proposed in the experimental chapter and to enable quantitative, automatic measurements and image processing. The key issues are to make the methodology simple and automatic, without decreasing its ability to follow dynamic changes occurring in the tear fluid. The development of a new image-processing software, standardization of the amount of fluid to be instilled and automatization of measurements should facilitate the clinical application of TCR assessment. Future studies should focus on development of standardized methodology to assess TTR as a clinical test. Correlations of OCT-based TCR with other tear film measures and fluorophotometric technique can lead to better understanding of the complexity of tear film dynamics and the role of tear clearance in supporting DED diagnosis.

CHAPTER III. LONGITUDINAL STUDY OF BIOMARKERS' TRENDS[2]

The following chapter provides a comprehensive description of the study protocol, methodology and results obtained over the time-course of the longitudinal prospective study of biomarkers' trends in contact lens wearers. In this longitudinal study the aim was to track the trends of the proposed measures of ocular physiology. The measures proposed in ***Chapter II*** were used as additional DED biomarkers and were followed parallelly with other ocular measures used commonly for supporting DED diagnosis.

3.1. ACKNOWLEDGEMENTS

Part of this longitudinal study regarding tear osmolarity changes in contact lens wearers was registered as a clinical trial on ClinicalTrials.gov - Protocol Registration and Results System, where it was published in May 21st, 2018 with clinical trial ID: NCT03531346.

[2] This study has been conducted in cooperation with Maryam Mousavi, MOptom from Biomedical Signal Processing group of Wroclaw University of Science and Technology. Some of the data reported below may constitute her doctoral dissertation.

3.2. METHODOLOGY

3.2.1. Study protocol development

Study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants after the nature and possible consequences of the study were explained.

Some important aspects and constraints have been considered while developing the study protocol for this longitudinal study. Based on the comprehensive literature review, practical experience, guidelines and consultations with eye care professionals, the most appropriate ocular measures to fulfil the definition of DED and its sub-classifications were chosen, considering the devices available in the laboratory and having in mind their clinical applicability. These ocular measures were described in sufficient details in ***Chapter I***.

To observe changes in ocular physiology subjects were fitted or re-fitted with soft contact lenses. The protocol was prepared considering the time and cost limits of the EDEN project. Free supply of lenses aided attendance outcomes, following a systematic schedule and minimized the number of drop-outs. The number of visits and the duration of the whole longitudinal study had to be balanced with the allocated funds. The executive part of the EDEN project was time-limited, hence the longitudinal study, including literature review, data analysis, protocol designing, experimental phase, practice, recruitment and scheduling, together with conducting the experiments and measurements were fitted within the allowed timeframe. A period of 13 months was estimated as the maximum period that gives the best balance between cost, subjects' availability and structure and timeframe of the EDEN project. It was hypothesized that this period of contact lens wear will somehow observably

impact ocular physiology. Daily disposable contact lenses were supplied to minimize the risk of inflammation, simplify contact lens care and increase subjects' adherence to the strict wearing protocol. By the time the measurements started, there were no data available on the long-term effect of modern daily disposable contact lens wear on ocular physiology. This study was the first independent investigation of the effect of daily disposable soft contact lenses on ocular physiology. The author is aware that the contact lens-induced DED differs in aetiology and pathophysiology from the typical multifactorial DED, however, supplying subjects with contact lenses seem to be the best available way to observe visible changes in the available timeframe and simultaneously increasing subjects' adherence to the study, without putting them at risk. At first, supplying subjects with artificial tears to observe changes in ocular physiology was considered, however this approach could probably meet with much lower rate of subjects' adherence and could have induced only a short-term effect. Additionally, the choice of artificial tears solution could be problematic, considering that there was no available gold standard in prescribing and choosing optimal topical solution for the subjects, at the time when this study protocol was being developed. In contrary, while fitting contact lenses, one can follow a protocol and choose the optimal lens from the available options.

Having in mind that even the non-invasive tests used for DED management require some alternation of blinking or bright illumination, it is important to proceed from the least to the most invasive measurements[223]. Evaluation sheets have been prepared following this rule. Therefore, each visit starts from filling-in the questionnaires, tear meniscus height *en face* evaluation and impedance-based measurements of tear osmolarity. These are followed by less invasive tests which utilize infrared radiation (NIKBUT) or do not require dye instillation (lipid layer thickness and bulbar redness scoring). Ultimately, measurements

utilizing topically applied solutions (TCR calculation) or vital dyes (ocular surface staining and FBUT estimation) are performed and the whole procedure is finished with the most invasive tests, which require eyelid inversion (LWE grading and infrared meibography).

The evaluation sheets for each of the visits are provided in the ***Appendices***. Each evaluation sheet describes the chronological order in which the measurements were performed. Figure 22 schematically represents the established timeline of the longitudinal study. As can be seen, this study can be divided into two phases - the longitudinal study and experimental phase.

The experimental phase was the time-frame during which all the experiments described in ***Chapter II*** were performed. This phase was partially coinciding with the Baseline part of the longitudinal study and partially comprised of the subjects participating in it.

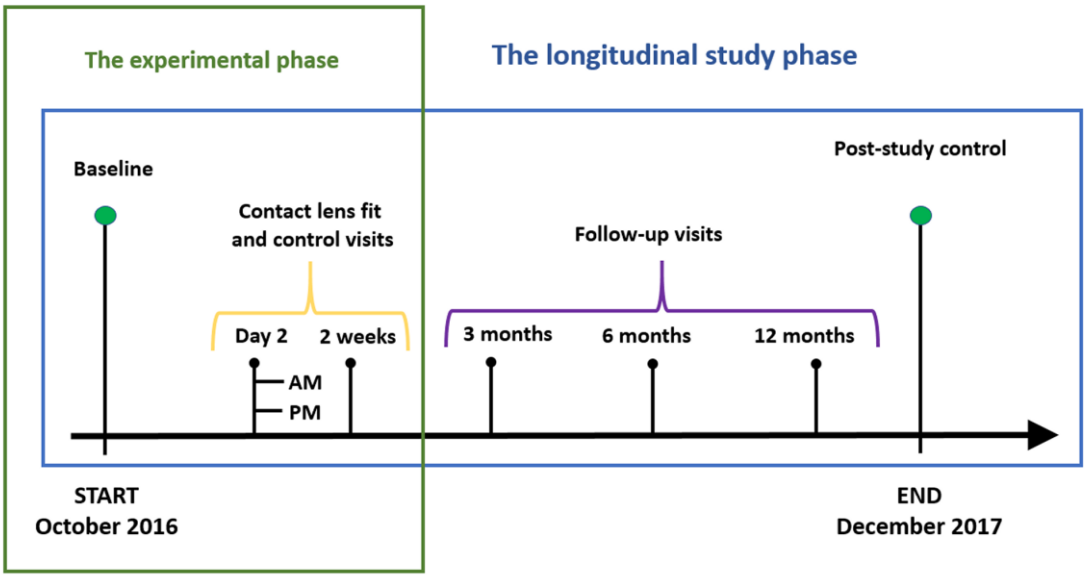


Figure 22. The schematic timeframe of the longitudinal study of biomarkers' trends

The longitudinal study comprised of two parts - the contact lens fitting part and the follow-up period. During the contact lens fitting part (marked in yellow) subjects were refitted with daily disposable soft contact lenses and the contact lens performance was assessed after four hours and two weeks of contact lens wear. The follow-up period (marked in purple) consisted of three follow-up visits at three, six and 12 months post-refitting. In the beginning of the longitudinal study, the Baseline visit was performed on subjects, who refrained from wearing their habitual contact lenses for at least 3 days prior to attending. After the study, subjects were advised to refrain from wearing contact lenses again for a period of 3 days and the post-study Control visit was performed. This was to compare the bare eye Baseline results with the Control bare eye results and to test whether changes potentially occurring during the 12-month follow-up period will lessen after refraining from contact lens wear.

The duration of each visit was time-limited. Subjects at Baseline visit were tested in the morning, which allows only two to three such visits per day, considering the time for the subjects to wake up and the overlapping schedules of the subjects attending the following visits for contact lens fit. The time of each visit was estimated and limited to one hour. Availability of the laboratory, national and academic holidays and weekends also were considered, when developing the study schedule. For the first phase of the measurements (marked in yellow) not to overlap with other follow-up visits (marked in purple), the second follow-up phase was scheduled three months post contact lens refitting. It allowed a break between visits to be as short as possible for a given sample size and some additional time for the data analysis.

Table 8 summarizes the study protocol.

Table 8. Summary of the longitudinal study protocol

Ocular measure	Baseline	Day 2	2-week	3-month	6-month	12-month	Control
Contact lens fit	×	✓	×	×	×	×	×
Contact lens fit control	×	✓	✓	✓	✓	✓	×
Medical history	✓	×	×	×	×	×	×
OSDI, DEQ-5	✓	×	✓	✓	✓	✓	✓
TMH_{K5M}	✓	×	✓	✓	✓	✓	✓
D-TMH, D-TMD and D-TMA	✓	×	✓	✓	✓	✓	✓
Tear osmolarity	✓	×	×	✓	✓	✓	✓
NIKBUT	✓ (PC)	✓(PL)	×	✓ (PL)	✓ (PL)	✓ (PL)	✓ (PC)
Ocular redness	✓	✓	✓	✓	✓	✓	✓
LLT	✓	✓	✓	✓	✓	✓	✓
Ocular surface staining with vital dyes	✓	✓	✓	✓	✓	✓	✓
CCT	✓	✓	✓	✓	✓	✓	✓
TCR	✓	×	×	✓	✓	✓	✓
Meibography	✓	×	×	✓	✓	✓	✓
LWE scoring	✓	×	×	✓	✓	✓	✓

NIKBUT – non-invasive Keratograph tear film break-up time; LLT – lipid layer thickness; CCT – central corneal thickness; TMH_{K5M} - tear meniscus height assessed with K5M; D-TMH - dynamic tear meniscus height, D-TMD - dynamic tear meniscus depth, D-TMA - dynamic tear meniscus area; LWE – lid wiper epitheliopathy score; TCR – tear clearance rate; PC - measurements performed on pre-corneal tear film; PL - measurements performed on the pre-lens tear film

Summarizing, the longitudinal study protocol consisted of qualifying visit (Baseline), contact lens fitting visit (following day - Day 2 visit), control visit at two weeks and follow-up visits at three months, six months and 12 months post-refitting, followed by the visit for the post-study assessment after three days (Control).

Laboratory temperature (°C) and humidity (%RH) were monitored with a thermo-hygrometry device (C3121, Comet, Czech Republic) and noted for each of the subjects. Subjects had an environmental adjusting period if they arrived at the laboratory directly

from outdoors. At each of the visits (except of Day 2) OSDI questionnaire was filled-in by the participants to distinguish symptomatic subjects and score ocular symptoms experienced during the last week preceding each visit.

3.2.2. Sample size calculation

Clinical differences to detect for the most common DED measures were based on the DEWS II - Diagnostic Methodology Report[25] and the resulting sample size calculations were based on 2-sample T-test comparison with 80% power and $P < 0.05$ significance level. The minimum sample sizes to detect the clinical difference in ocular measures were estimated. In more complex experiments, that require repeated measures analysis of variance (ANOVA), it is better to consider the number of degrees of freedom (based on both the number of treatments/visits and the number of replicates), having in mind that it is recommended to have at least 15 such degrees. Additionally, as DED measures often deviate from a normal distribution, it is advised to increase the sample size by 10% in order to compensate for this deviation[25]. Considering all the criteria mentioned above, and when sufficient data was available in literature regarding the data variability, the minimum necessary sample size was estimated for each of the considered measures.

The minimum sample size was estimated as 40 subjects. Considering potential drop-outs and the time and cost limit, the maximum sample size was estimated to be around 60 for a 12-month period of observation. However, increasing the number of the recruited subjects from 40 to 60 would only have a small impact on the power of the statistical analysis, as the number of degrees of freedom is already substantial when sample size equals 40.

3.2.3. Contact lens fit

Several studies have been conveyed to investigate a long-term impact of soft contact lens wear on ocular physiology[224-228]. They were generally focused on different lens designs, oxygen permeability[228] and their impact on the ocular surface morphology[224,226,229], tear stability or ocular surface sensory function[225]. Day 2 visit of the longitudinal study was dedicated to fitting subject with contact lenses. This visit was divided into morning and afternoon sessions. In the morning subjects were fitted with two different types of daily disposable contact lenses: Silicon Hydrogel (SiHy, Delefilcon A) on the right eye and Hydrogel (Hy, Omafilcon A) on the left eye. After four hours of wear, further assessment was performed, including: subjective comfort assessment, contact lens fit, pre-lens NIKBUT measurements with K5M and ocular surface staining with fluorescein after lens removal. Contact lens fit was recorded on the corresponding evaluation sheet (see ***Appendix 6***). Newly-fitted lens was chosen based on the contact lens fit, reported subjective comfort after 4 hours of wear and pre-lens tear film surface quality. Tear film surface quality measurements were based on the quality of reflected Placido rings, as described in the study of Mousavi et al.[39]. Contact lens fit assessment included contact lens centration (decentration not greater than 0.2 mm in any direction), corneal coverage, horizontal lag (within 0.5–1.0 mm), blink movement (within 0.25–0.50 mm), push-up test (within 2–4 mm/s) and the binocular corrected visual acuity rating for distance and near vision[230,231]. Lenses were inserted straight from their blister packs and were worn for approximately four hours before fit assessment. Based on these criteria, one lens was chosen for the subject to wear it for the whole duration of the study. Subjects were given written instructions on contact lens care and hygiene, which they were obliged to follow (see ***Appendix 7***). Moreover, all contact lens novices and some other volunteers were scheduled for an

additional individual visit with an eye-care professional, when they were introduced to contact lens handling, putting the lenses on and taking them off properly, contact lens hygiene and adverse effects of contact lens misuse.

3.2.4. Ocular symptoms assessment

Ocular symptoms usually accompany DED thus, they were adapted in its most recent definition as one of the fundamental characteristics of DED. While in severe cases of the disease, ocular symptoms are evident, it is not always the case for its mild and early manifestations. It was estimated, that more than one-third of contact lens wearers experience DED symptoms with contact lens wear[232,233] and that discomfort accounts for around 51% of contact lens dropouts[234].

DED symptoms were assessed with DEQ-5[235], while OSDI[236] was used to assess general ocular surface disease symptoms over the time-course of the longitudinal study. OSDI is the most widely used questionnaire for the assessment of ocular symptoms in clinical trials. It measures the frequency of symptoms, environmental triggers, visual disturbance and vision related quality of life[237]. Recently, many other questionnaires have established concurrent validity against the OSDI. The consensus is to use the OSDI[238-243] due to its strong establishment in the field or the DEQ-5[243] due to its short length and discriminative ability[25,235]. Distributions of currently available metrics, including signs and symptoms of DED fluctuate over time and vary significantly within different levels of disease severity[8,9,25]. In this study, a combination of OSDI and DEQ-5 questionnaires (see ***Appendix 2***) was used to assess ocular symptoms throughout the time-course of the study. Questionnaires were self-administered by the subjects. Since the assessment

of contact lens-related discomfort was not in the scope of this study, a standard CLDEQ-8 questionnaire was not used to assess contact lens-related dry eye symptoms[244].

3.2.5. Tear osmolarity

Tear osmolarity was measured from the inferior lateral tear meniscus in both eyes of each subject with use of the chip-based osmometer (TearLab® Osmolarity System, TearLab Corp, San Diego, CA, USA). Electronic check cards calibration of the instrument was performed daily, based on the manufacturer's guidelines. The control solution calibration was performed every time a new box of test cards was opened. The device was powered for the whole duration of the study and was kept fully charged. The device and the test cards were stored in the laboratory in which the measurements were conducted, to ensure them being in the same temperature as the environment. The same diagnostic pen was used for all the measurements. The tear osmolarity of the right eye of each subject was always measured first. Subjects were asked to sit with their chin tilted slightly upward and eyes directed toward the ceiling. The tip of the pen was positioned just above the lower eyelid and gently applied to the lower tear meniscus. Care was taken not to induce reflex tearing, not to touch the globe or pressing the eyelid during collection. After the successful sampling and docking of the diagnostic pen, values were displayed on the device and then recorded on the corresponding evaluation sheet (see **Appendix 5, 8, 9 and 10**). Two measurements for each eye were performed and the obtained results were averaged[74]. If the intra-eye results differed by more than 9 mOsm/L, the tear osmolarity was assessed three times per eye to obtain more reliable averaged estimate of tear osmolarity for that eye. The tear osmolarity was assessed during the Baseline visit and followed at 3-month, 6-month, 12-month and Control visit.

3.2.6. Non-invasive Keratograph tear film break-up time

Measurements of non-invasive Keratograph 5M tear film break-up time (NIKBUT) were performed according to the manufacturer's instructions. The measurements were performed in mesopic conditions. As mentioned before, measurements of NIKBUT with K5M are performed under infrared (880 nm) Placido disk illumination. Subjects were asked to blink twice naturally to reconstitute the tear film, while fixating on the central target and keeping their eyes open for as much time as they felt comfortable. The video-recording of the pattern starts automatically after two blinks and the pattern can be observed in real time. Break-ups are automatically detected by K5M and appear on a polar-type grid representing the corneal area, as shown in the Figure 23. The video recording with K5M lasts until subject next blink. The K5M algorithm for estimating tear film quality is proprietary. Therefore, two numerical values are provided at the end of each assessment:

- First-NIKBUT (F-NIKBUT), which is the time in seconds taken from the last blink to the first appearance of a substantial deformation of the Placido rings;
- Mean-NIKBUT (M-NIKBUT), which is the average of the time taken from the last blink to the Placido ring deformations in all the regions monitored over the duration of the recording. Three measurements per eye were acquired, starting always from the right eye and alternated.

F-NIKBUT and M-NIKBUT were noted on the corresponding evaluation sheet. NIKBUT was measured at Baseline, Day 2, 3-month, 6-month, 12-month and Control visit (see ***Appendix 5, 6, 8, 9 and 10***). It is important to notice that pre-corneal NIKBUT and pre-lens NIKBUT were assessed at different days/times. The pre-corneal NIBKUT was inspected during the Baseline and Control visit, whereas the pre-lens NIKBUT at follow-

up visits. NIKBUT estimates measured at Day 2 were used for contact lens fitting assessment, as proposed by Mousavi et al.[39]. Figure 24 shows exemplary acquisitions with K5M, corresponding to different subjects with two different levels of tear film surface quality.

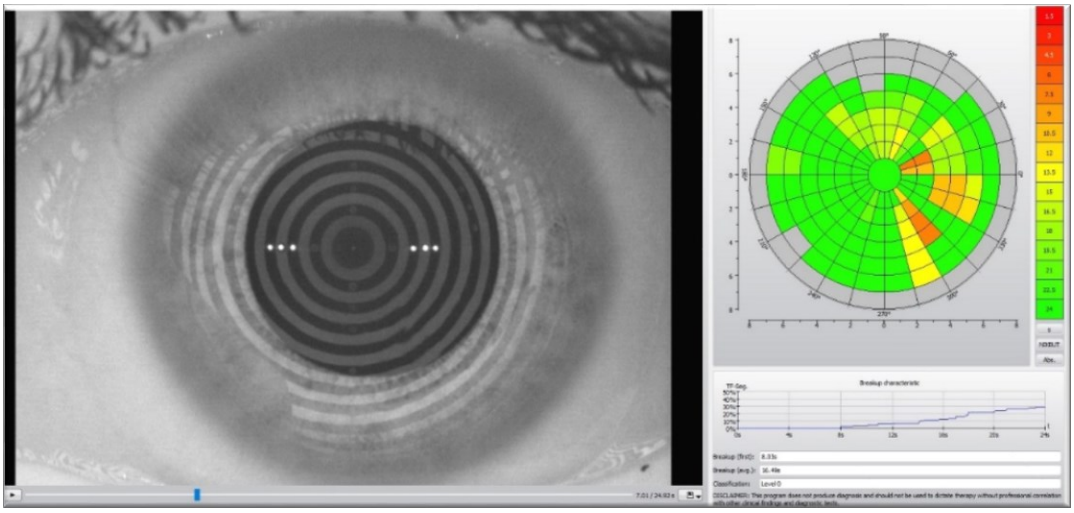


Figure 23. Interface of the K5M for NIKBUT measurements. Left panel shows an image of the eye with the superimposed Placido rings from a 25-second sequence, whereas the right panel shows the distortion map of the rings and the respective estimated break-up times

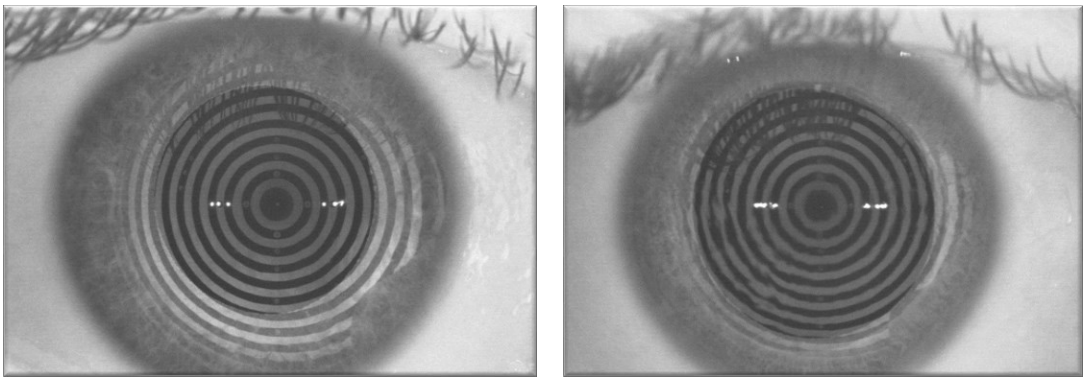


Figure 24. Exemplary images of the Placido disks reflection observed with K5M on the eyes without contact lenses. Left: good tear film surface quality - no distorted Placido rings; Right: visible tear film break-ups, low tear film surface quality and distorted Placido rings

3.2.7. Lipid layer thickness

Tear film lipid layer can be investigated with thin layer interferometry-based techniques. Colour and brightness of the interference images are analysed to yield lipid layer thickness[245-249]. The thickness of the lipid layer has been reported to be from 15 to 157 nm, with a mean of approximately 42 nm[245]. Lipid layer has been found to play an important role in tear film stability[17,250]. The evaluation of lipid layer thickness (LLT) with K5M is achieved based on the phenomena of thin-film interference. It is a physical phenomenon in which light waves reflected by the upper and lower boundaries of the thin layer interfere with one another, either enhancing or reducing the reflected light. When the thickness of the film is an odd multiple of one quarter-wavelength of the light projected on it, the reflected waves from both surfaces interferometrically cancel each other. Since the wave in not reflected, it is transmitted instead. When the thickness is a multiple of a half-wavelength of the light, the two reflected waves reinforce each other, increasing the reflection and reducing the transmission. Therefore, when white light consisting of a range of wavelengths is being projected on the thin lipid layer of the tear film, certain wavelengths (colours) are intensified while others are attenuated. The colour pattern depends on the regional LLT. It has been suggested that these interference patterns could be used to observe the fluidity and thickness of the lipid layer between blinks[251,252].

The *Lipid Layer* function of K5M's software was utilized to assess LLT based on the abovementioned phenomenon. To do so, the magnification was switched to ×1.4, which enables an observation of subtle changes in the interference pattern and the debris floating over the surface of the tear film. K5M head was being moved in small increments to focus on the upper thin layer of the tear film. The most effective way to achieve this is to bring

the Placido rings into focus on the corneal surface and subsequently to slowly pull the camera away to slightly defocus the Placido rings, bringing the lipid layer into depth of focus. Once it is achieved, the colourful thin-film interference pattern and small particles floating in the lipid layer can be observed and be recorded. Subjects were advised to look at the central focusing spot of the device and blink freely. After pressing the *Rec* button, the video of the lipid layer movement after the blink was recorded for a duration of three non-forceful consecutive blinks. The images were stored automatically, then exported and saved on a portable device for further analysis. This procedure was performed for each eye of the subject at Baseline, 3-month, 6-month, 12-month and Control visit. Successfully done procedure was noted on the evaluation sheet (***see Appendices 5, 8, 9, 10***). Lipid layer thickness based on these recordings was evaluated qualitatively by masked practitioner. Based on the observed pattern it was scored as '0' when lipid layer was normal, '-1' when the colourless reflections were suggesting a thin lipid layer and '+1' when the lipid layer was reported as thicker than normal and an evident increase in colourful spots in the lipid layer could be observed. Examples of different lipid layers acquired for two of the subjects and their scores are displayed in the Figure 25.

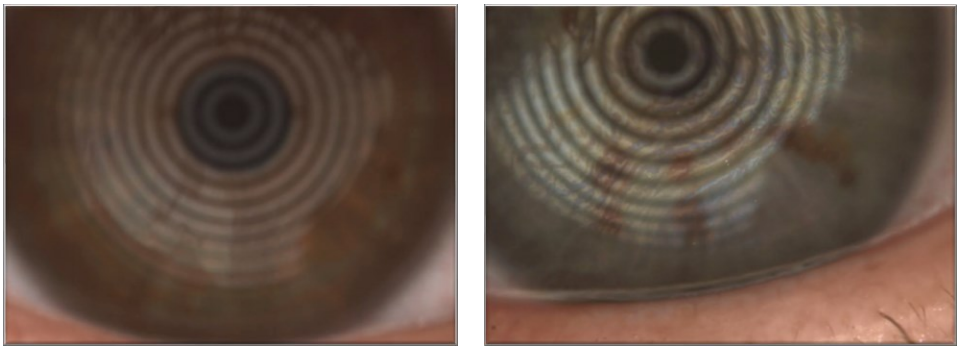


Figure 25. Exemplary frames from the video of the lipid layer acquired for two of the subjects with K5M. Left: score '-1' - colourless reflections suggesting a thin lipid layer; Right: score '+1' - an increase in colourful fringes indicating thicker lipid layer

3.2.8. Ocular redness

Bulbar and limbal redness were recorded by *R-Scan* function of the K5M's software called *Bulbar redness.* Default settings were used. The images were stored and analysed automatically. To monitor the ocular surface redness, the camera was aligned so that the grey focusing disc covered the iris of the subject. Subsequently subjects were asked to open their eyes as wide as possible and the image was captured. Figure 26 shows an exemplary image of the eye assessed with the *Bulbar redness* function.

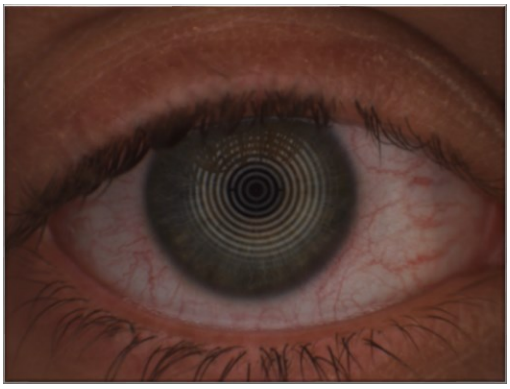


Figure 26. An exemplary image acquired for one of the subjects with K5M for automatic assessment of bulbar and limbal redness

The device automatically grades bulbar and limbal redness of the temporal and nasal area of the exposed ocular surface. This procedure was performed at Baseline, 3-month, 6-month, 12-month and Control visit. The nasal and temporal values of the bulbar and limbal redness of both eyes were noted on the corresponding evaluation sheet (see ***Appendix 5,9 and 10***) and averaged for each eye as bulbar and limbal redness, respectively.

3.2.9. Ocular surface staining with fluorescein

Corneal and conjunctival staining with fluorescein was performed to assess ocular surface damage and to ensure no adverse effects of contact lens wear over the time-course of the longitudinal study. Staining was examined with a slit lamp biomicroscope, with ×16 magnification, cobalt blue illumination and a Wratten 12 yellow-barrier filter. Efron's grading scheme was utilized to assess the severity of staining of the cornea and conjunctiva[93] and the corresponding scores were noted on the evaluation sheet (see ***Appendix 5, 6, 8, 9 and 10***).

After grading the ocular staining by an optometrist with the slit-lamp, images of the staining were captured with the K5M's tool called *Fluo-Imaging* and stored automatically for further inspection. Default settings were used. Figure 27 demonstrates two exemplary images of the ocular surface stained with fluorescein, acquired for one of the subjects with the use of the *Fluo-imaging* tool of K5M.

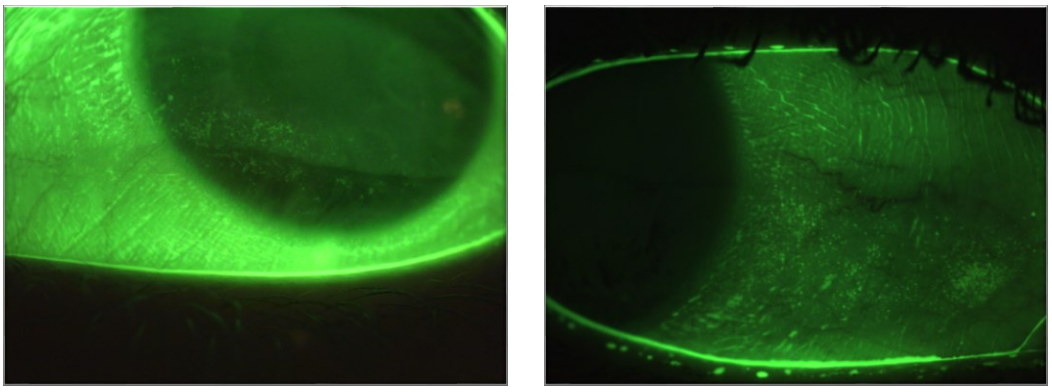


Figure 27. Staining with fluorescein of the ocular surface, acquired for one of the subjects with Fluo-imaging built-in software of K5M

Subjects were asked to look at the red central dot of the device. One image of the central ocular surface was captured per eye, to visualise corneal staining. To do so, the reflection

of the red target on the ocular surface must be put into focus. Subsequently, to assess conjunctival staining, subjects were asked to look up and then alternately to their left and right side. For each of the gaze positions, one image of the conjunctival staining was captured. The procedure was applied to both eyes of each subject.

3.2.10. Ocular surface staining and lid wiper staining with lissamine green

To perform LWE scoring and asses conjunctival staining, 1.5 mg lissamine green strip (HUB Pharmaceuticals, LLC, Scottsdale, Arizona) was moistened with 0.9% buffered saline solution. Excess liquid was not shaken from the strip. After advising the subject to look away and after gently pulling down his/her lower eyelid, the lissamine green strip was gently inserted into the lower conjunctival sac, with care taken not to touch the bulbar conjunctiva with the strip. After instillation, the retracted lower eyelid was slowly released, and this procedure was repeated for the other eye. Subsequently, subjects were advised to keep their eyes closed for one minute. Next, the staining was recorded by *Imaging* tool of K5M's software. Magnification was switched to ×0.5, which enabled simultaneous observation of both tarsal and nasal bulbar conjunctiva. While subjects were asked to fixate on a central target, the K5M camera was focused, and one static image of the whole exposed ocular surface was obtained. When deep conjunctival staining was apparent on a relatively small area, the camera was switched to higher magnification to closely investigate the staining in that area. Figure 28 shows an exemplary image of lissamine green staining of the conjunctiva acquired for one of the subjects and the corresponding magnified image.

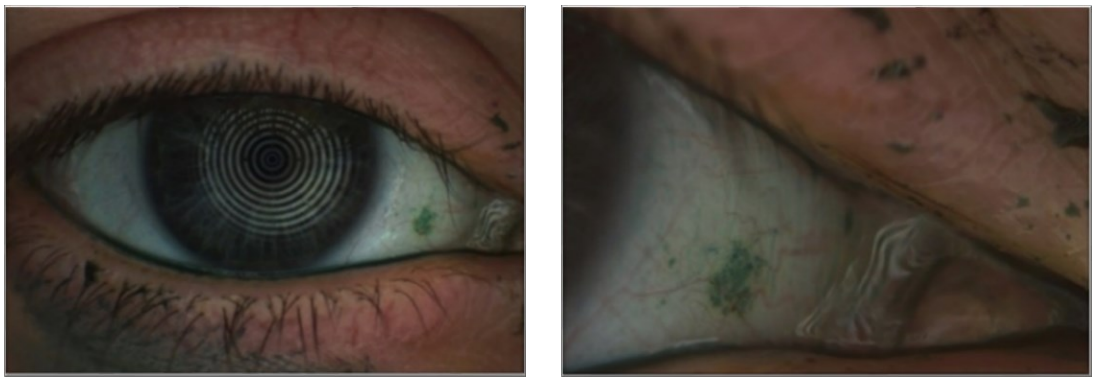


Figure 28. Lissamine green staining of the conjunctiva (left) and the corresponding close-up (right) acquired for one of the subjects with K5M

For each subject, at least one image of the exposed anterior eye surface was recorded, while the eye was kept in a primary gaze position. The images were stored automatically and Efron's grading scale was used to assess the severity of staining.

Subsequently, right after careful eyelid inversion, an image of the lid wiper staining was captured. Since the lid inversion can be uncomfortable to the subjects, the Meibography image was acquired right after the image of the lid wiper was taken, while the subject's eyelid was still inverted. Figures below show exemplary images of the inverted upper (Figure 29) and lower (Figure 30) eyelid - one, with no staining (or with Marx's line only) and one with visible lid wiper staining, suggestive of LWE. Staining scores were assessed independently by two practitioners and graded in accordance with the scheme proposed by Korb et al.[95] based on the horizontal length and sagittal width of the staining. When only the Marx's line or no staining was observed, the corresponding score was marked as '0', suggesting the eye without LWE.

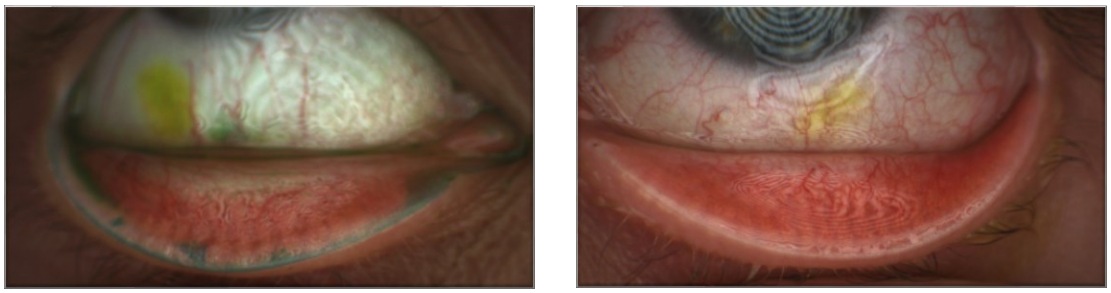


Figure 29. Upper eyelid lid wiper staining suggestive of LWE (left, score 3) and Marx's line (right, score 0), acquired with K5M for two different subjects

Lid inversion for LWE scoring, as well as for Meibomian glands drop-out scoring (Meibo-score) were performed at Baseline, 3-month, 6-month, 12-month and Control visit (see ***Appendices 5, 9 and 10***).

Figure 30. Lower eyelid lid wiper staining suggestive of LWE (left, score 3) and Marx's line (right, score 0), acquired with K5M for two different subjects

3.2.11. Infrared meibography

Meibomian glands were visualised in infrared with Meibo-Scan K5M tool called Meibography Single Image. Default settings were used, with ×0.5 magnification. Images were acquired for both upper and lower eyelid of both eyes of each subject. As mentioned before, the Meibography infrared image was acquired right after the white light image of the lid wiper was taken. To do so, the device was quickly switched to Meibography mode. Successful acquisition was reported on the corresponding evaluation sheet at Baseline, 3-month, 6-month, 12-month and Control visit (see ***Appendices 5, 9 and 10***). The images were stored automatically for further processing. One of the acquired images is displayed below in Figure 31.

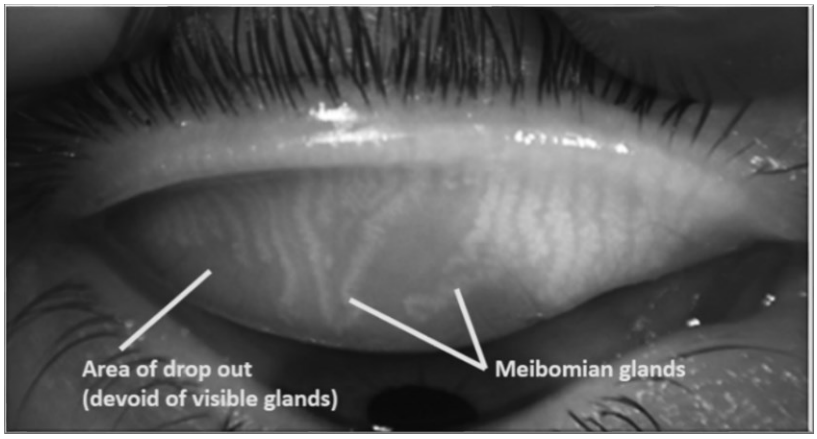


Figure 31. An exemplary meibography infrared image of the upper eyelid acquired for one of the subjects with K5M Meibography Single Image tool

ImageJ (US National Institutes of Health, Bethesda, MD, USA) image processing software was used to perform the image processing and to calculate the Meibo-score based on acquired infrared meibography. The *Polygon selection* tool of the software was used as shown in Figure 32. Firstly, the whole exposed tarsal area of the eyelid was outlined and

the surface area of that outline (in pixels) was assessed. Subsequently, this surface area was compared with the surface area (in pixels) of the zone devoid of Meibomian glands, which was also marked and calculated with the *Polygon selection* tool.

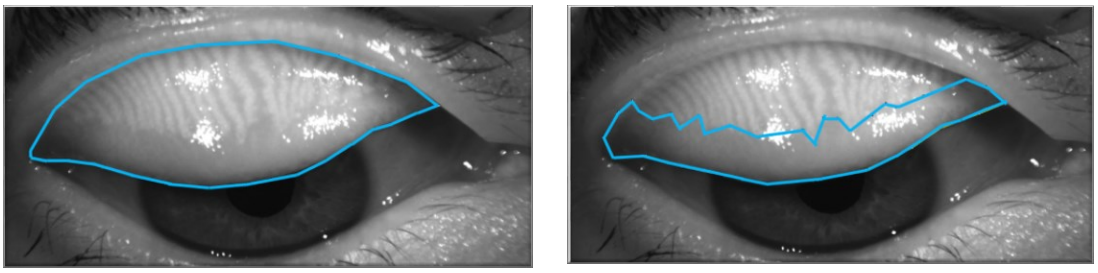
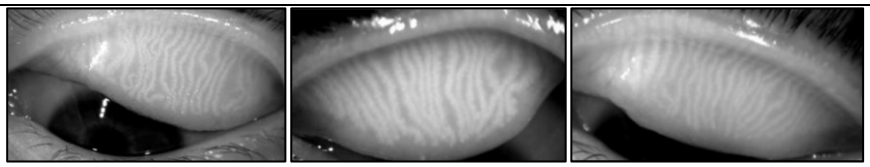
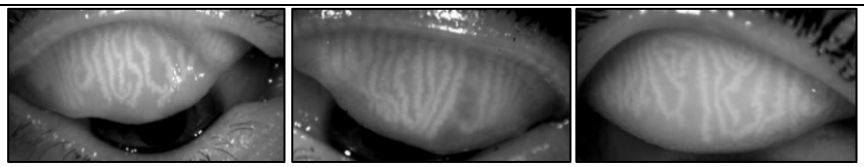
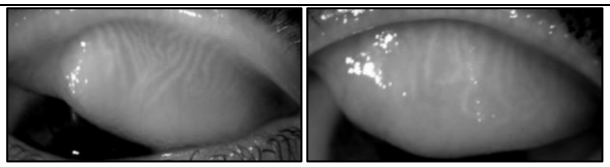


Figure 32. Meibomian glands image analysis performed with the Polygon selection tool of ImageJ; Left: Selection of the entire eyelid area; Right: selection of the area devoid of Meibomian glands; The lines of the outline were thickened and marked with bright colour for better visibility

Thus, the Meibo-score was calculated as a percentage of the Meibomian glands loss compared with the whole surface of the eyelid and scored by a masked evaluator.

Originally, to score the Meibomian gland loss, the *Meiboscale,* designed by Heiko Pult was utilized[253]. Each score of the Meiboscale is a natural number from 0 (no loss) to 4 (substantial loss) that corresponds to the percentage drop-out of Meibomian glands. Table 9 shows examples of different levels of Meibomian glands loss acquired with K5M as if they were scored based on the *Meiboscale*.

Table 9. Exemplary images of the upper eyelid acquired with K5M infrared Meibography tool and their corresponding scores developed by Pult et al.

Meiboscale	Exemplary images of the upper eyelid acquired with K5M
Score 0 (≈0% loss)	
Score 1 (≤25% loss)	
Score 2 (26-50% loss)	
Score 3 (51 - 75% loss)	
Score 4 (>75% loss)	**No subjects reported with this level of Meibomian glands drop-out**

Images were acquired for subjects taking part in the longitudinal study. The scale was proposed by Pult et al.254

3.2.12. Corneal thickness

Studies show that changes in corneal thickness may occur during contact lens wear. In this study central corneal thickness (CCT) was measured based on the scans acquired with OCT (Copernicus, Optopol, Poland) with the use of built-in software. Dedicated lens system was attached to the OCT to visualize the anterior segment of the eye. The anterior segment module of the device allows corneal and anterior imaging with a resolution of 3 microns. 'Asterisk' anterior eye scan protocol was used and the number of cross-sections

of the cornea was put on maximum value. CCTs were displayed automatically and noted on the corresponding evaluation sheet at Baseline, 2-weeks, 3-month, 6-month, 12-month and Control visit (see ***Appendices 5, 8, 9 and 10***).

3.2.13. Statistical Analysis

Group mean, median values, standard deviations and ranges of the ocular biomarkers assessed over the time-course of the longitudinal study are displayed in the ***Results*** sub-section of this chapter, together with the reported statistically significant differences between different groups of subjects. Non-parametric two-way ANOVA (Friedman test) was used to assess statistically significant changes between Baseline and 12-month visit. Subsequently, post-hoc comparisons for each pair of visits was conducted, followed by comparing Baseline and Control visit (bare eye) results. The post-hoc analysis was conducted with the Wilcoxon signed rank test. Additionally, linear correlations between all the measures collected over the time-course of the longitudinal study were tested. There is also a question whether to correct the *P*-values obtained for the number of tests made (to use 'Bonferroni correction'). Armstrong et al.[255] argued that the use of the Bonferroni correction should depend on the circumstances of the study. It should not be used routinely and should be considered if:

- a single test of the 'universal null hypothesis' (H_0) that all tests are not significant is required;
- it is imperative to avoid a type I error, i.e. claiming a significant result when it is absent;
- many tests are carried out without pre-planned hypotheses.

None of these circumstances were characteristic to this study and therefore the Bonferroni correction was not applied.

3.3. RESULTS

This section summarizes the results obtained throughout the time-course of the longitudinal study and the results of the performed statistical analysis, together with the description of the subjects and the criteria based on which the subjects were divided into groups.

3.3.1. Subjects and contact lens fit

Fifty-five subjects (36 females and 19 males) participated in the study for its whole duration. Before commencing the study, six subjects were contact lens neophytes, 30 wore monthly, 13 wore fortnightly and six wore daily disposable soft contact lenses habitually. A clear majority of the subjects (N = 48) wore SiHy lenses before commencing the study. The group mean age was (mean ± standard deviation) 26 ± 4 y/o and was ranging from 20 to 37 y/o. Corrected visual acuity of all subjects was stable over the time-course of the study and was ranging from 0.0 to –0.1 logMAR. Subjects were fitted with contact lenses based on the procedure performed at Day 2 and described by Mousavi et al.[39]. Thirty-eight subjects were fitted with SiHy (25 females and 13 males) and 17 subjects (11 females and 6 males) were fitted with Hy daily disposable soft contact lenses (see Figure 33).

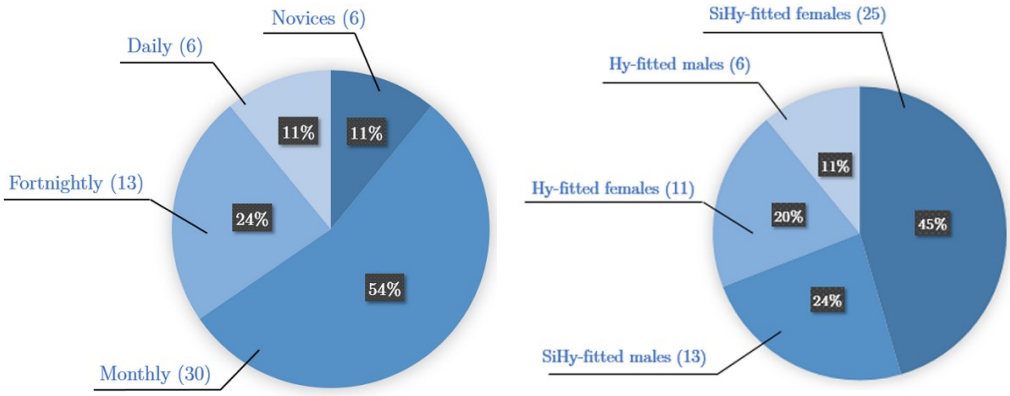


Figure 33. Habitual contact lenses used by the subjects before participating in the study (left) and post-refitting subjects' demographics (right)

There was no statistically significant difference in age (P = 0.978) and gender distribution (P = 0.797) between the SiHy and Hy-fitted group, as reported with Mann-Whitney test. Statistical significance (P-values) of the differences between right and left eye during Baseline visit is shown in Table 10.

Table 10. Inter-eye differences in the ocular measures assessed during the baseline visit

Ocular measure	**Difference between OD and OS**	**Ocular measure**	**Difference between OD and OS**
M-NIKBUT	P = 0.469	**Corneal staining**	P = 0.313
F-NIKBUT	P = 0.331	**Conjunctival staining**	P = 0.736
TMH_{K5M}	P = 0.900	**FBUT**	P = 0.437
Tear osmolarity	P = 0.600	**Upper LWE score**	P = 0.598
Ocular Redness – Bulbar	P = 0.923	**Lower LWE score**	P = 0.556
Ocular Redness – Limbal	P = 0.758	**Upper lid Meibo-score**	P = 0.888
Lipid layer thickness	P = 0.907	**Low lid Meibo-score**	P = 0. 379

M-NIKBUT - Mean non-invasive tear film break-up time; F-NIKBUT - First non-invasive tear film break-up time; TMH_{K5M} - K5M-based tear meniscus height, FBUT - fluorescein tear film break-up time; OD - right eye, OS - left eye; LWE - lid wiper epitheliopathy

Wolffsohn et al. demonstrated that soft contact lens movement and ocular surface indentation can be influenced by both lens edge design and midperipheral lens profile[224]. Two lenses used in the longitudinal study share comparable midperipheral characteristics. Images of the lens peripheral shapes and edge profiles, acquired with OCT for the lenses of the same refractive power were displayed in the Figure 34. The images were resized and cropped to show the area of interest.

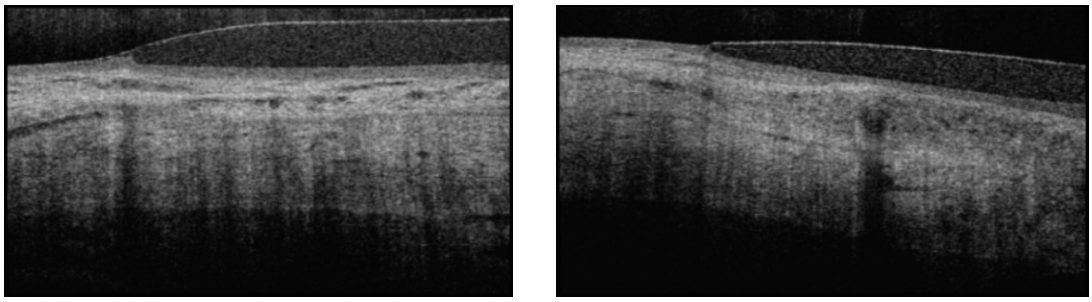


Figure 34. Lens edge of hydrogel lens (left part) and silicone-hydrogel lens (right part) used in the study. Images acquired with OCT. Images were resized and cropped. Lenses presented here were of the same refractive power

Differences (*P*-values) in ocular measures between SiHy and Hy-fitted group are displayed in Table 11. Except for one ocular measure (TMH-based TCR at Control visit), there were no statistically significant differences noted between SiHy-fitted and Hy-fitted group over the time-course of the study. This may indicate that both lenses achieved similar performance and had comparable impact on ocular physiology.

Table 11. Statistical significance (P-values) of differences between SiHy and Hy-fitted group in ocular measures reported over the time-course of the longitudinal study

Ocular measure	Baseline	3-month	6-month	12-month	Control
Temperature	$P = 0.654$	$P = 0.252$	$P = 0.553$	$P = 0.441$	$P = 0.139$
Humidity	$P = 0.662$	$P = 0.071$	$P = 0.524$	$P = 0.668$	$P = 0.207$
OSDI	$P = 0.964$	$P = 0.547$	$P = 0.622$	$P = 0.396$	$P = 0.376$
DEQ-5	$P = 0.380$	$P = 0.905$	$P = 0.207$	$P = 0.621$	$P = 0.778$
M-NIKBUT	$P = 0.222$	$P = 0.629$	$P = 0.348$	$P = 0.629$	$P = 0.909$
F-NIKBUT	$P = 0.566$	$P = 0.695$	$P = 0.848$	$P = 0.689$	$P = 0.810$
Tear osmolarity	$P = 0.282$	$P = 0.080$	$P = 0.702$	$P = 0.433$	$P = 0.688$
TMH_{K5M}	$P = 0.074$	$P = 0.110$	$P = 0.137$	$P = 0.160$	$P = 0.056$
TCR_{TMH}	$P = 0.051$	$P = 0.991$	$P = 0.138$	$P = 0.989$	$\mathbf{P = 0.026}$*
TCR_{TMD}	$P = 0.058$	$P = 0.620$	$P = 0.339$	$P = 0.779$	$P = 0.719$
TCR_{TMA}	$P = 0.068$	$P = 0.294$	$P = 0.108$	$P = 0.401$	$P = 0.133$
D-TMH	$P = 0.588$	$P = 0.184$	$P = 0.653$	$P = 0.604$	$P = 0.984$
D-TMD	$P = 0.949$	$P = 0.157$	$P = 0.887$	$P = 0.636$	$P = 0.535$
D-TMA	$P = 0.582$	$P = 0.814$	$P = 0.887$	$P = 0.825$	$P = 0.366$
Bulbar Redness	$P = 0.406$	$P = 0.742$	$P = 0.315$	$P = 0.547$	$P = 0.825$
Limbal Redness	$P = 0.891$	$P = 0.453$	$P = 0.085$	$P = 0.898$	$P = 0.879$
Lipid layer thickness	$P = 0.120$	$P = 0.214$	$P = 0.744$	$P = 0.694$	$P = 0.900$
Corneal staining	$P = 0.977$	$P = 0.218$	$P = 0.181$	$P = 0.655$	$P = 0.682$
Conjunctival staining	$P = 0.598$	$P = 0.422$	$P = 0.649$	$P = 0.244$	$P = 0.695$
FBUT	$P = 0.688$	$P = 0.137$	$P = 0.949$	$P = 0.152$	$P = 0.984$
Upper LWE score	$P = 0.779$	$P = 0.132$	$P = 0.453$	$P = 0.574$	$P = 0.796$
Lower LWE score	$P = 0.294$	$P = 0.872$	$P = 0.668$	$P = 0.350$	$P = 0.979$
Upper lid Meibo-score	$P = 0.906$	$P = 0.133$	$P = 0.494$	$P = 0.646$	$P = 0.248$
Lower lid Meibo-score	$P = 0.662$	$P = 0.848$	$P = 0.466$	$P = 0.523$	$P = 0.642$
CCT	$P = 0.721$	$P = 0.440$	$P = 0.344$	$P = 0.549$	$P = 0.442$

*OSDI - ocular surface disease index; DEQ-5 - 5-item dry eye questionnaire; M-NIKBUT - mean non-invasive tear film break-up time; F-NIKBUT - first non-invasive tear film break-up time; TCR - tear clearance rate; D-TMH - tear meniscus height; D-TMD - tear meniscus depth; D-TMA - tear meniscus area; LWE - lid wiper epitheliopathy; CCT - central corneal thickness; * denotes statistically significant difference*

Additionally, as there were no statistically significant differences in any of the assessed ocular measures between right and left bare eye and between SiHy and Hy-fitted group, all subjects were unified and analysed as one cohort of re-fitted contact lens wearers. In some cases, the division of subjects into groups was performed with respect to the reported signs or symptoms of DED and their recommended thresholds.

3.3.2. Study environment

The group mean and median values, standard deviations and ranges of the laboratory temperature [°C] and relative humidity [%RH] over the time-course of the study were displayed in Table 12 and Table 13, respectively. Non-parametric two-way ANOVA showed statistically significant difference in temperature since baseline visit until 12 months ($\chi^2 = 63.66$, $P < 0.001$).

Table 12. Temperature in the laboratory during each of the sessions

Temperature [°C]						
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	24.3 ± 1.0	24.9 ± 1.0	24.1 ± 1.5	25.2 ± 1.0	23.5 ± 0.7	23.7 ± 1.2
Median	24.5	25.0	24.1	25.2	23.4	23.2
Range	[21.8, 26.3]	[22.5, 26.8]	[21.5, 26.6]	[22.1, 26.6]	[22.1, 24.9]	[21.8, 26.2]

SD- standard deviation

Post-hoc comparisons conducted for each pair of visits showed statistically significant differences in temperature between Baseline and other visits ($P = 0.005$, $P < 0.001$ and $P < 0.001$ for 2-week and 6-month and 12-month, respectively), 2-week and 3-month ($P = 0.025$), 2-week and 12-month ($P < 0.001$), 3-month and 6-month ($P < 0.001$), 3-month and 12-month ($P = 0.015$) and 6-month and 12-month visit ($P < 0.001$).

Additionally, Wilcoxon test showed statistically significant difference in temperature between Baseline and Control visit ($P < 0.001$). Non-parametric two-way ANOVA (Friedman test) showed statistically significant difference in relative humidity in the laboratory since Baseline until 12-month visit ($\chi^2 = 78.08$, $P < 0.001$).

Table 13. Relative humidity in the laboratory during each of the sessions

Relative humidity [%RH]						
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	32.1 ± 4.7	31.4 ± 4.9	25.4 ± 3.4	31.9 ± 7.9	34.8 ± 3.4	32.6 ± 3.5
Median	32.0	30.3	24.4	32.6	34.6	32.0
Range	[23.3, 44.4]	[22.3, 39.8]	[20.3, 33.2]	[18.5, 47.7]	[26.6, 45.1]	[28.7, 45.0]

SD - standard deviation; Relative humidity is the ratio of the partial pressure of water vapor to the equilibrium vapor pressure of water at a given temperature. Relative humidity depends on temperature and the pressure of the system

Statistically significant differences in relative humidity of the environment were noted between Baseline and 3-month ($P < 0.001$), Baseline and 12-month ($P < 0.001$), 2-week and 3-month ($P <0.001$), 2-week and 12-month ($P < 0.001$), 3-month and 6-month ($P < 0.001$), 3-month and 12-month ($P < 0.001$) and 6-month and 12-month visit ($P = 0.021$). Additionally, Wilcoxon test showed statistically significant difference in relative humidity between Baseline and Control visit ($P < 0.001$).

3.3.3. Reported symptoms - OSDI

The group mean, median values, standard deviations and ranges of OSDI reported over the time-course of the study were shown in Table 14. Friedman test showed statistically significant differences in OSDI over the time-course of the study, since Baseline until 12-month visit ($\chi^2 = 9.53$, $P = 0.049$). Subsequently, post-hoc comparisons for each pair of visits showed statistically significant difference in OSDI between Baseline and 2-week ($P = 0.006$), and 2-week and 6-month visit ($P = 0.045$). Additionally, statistically significant differences between Baseline and Control visit in OSDI were noted ($P = 0.002$).

Table 14. OSDI scores reported over the time-course of the study

	OSDI [-]					
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	13.9 ± 11.9	11.0 ± 12.8	11.3 ± 10.7	11.9 ± 9.1	12.6 ± 11.5	6.2 ± 9.5
Median	10.4	6.3	8.3	8.3	9.1	2.1
Range	[0.0, 47.7]	[0.0, 61.1]	[0.0, 52.1]	[0.0, 37.5]	[0.0, 59.1]	[0.0, 37.5]

OSDI – Ocular Surface Disease Index; Mild 13-22; Moderate 23-32; Severe ≥ 33 [25]; SD – standard deviation

Subsequently, subjects were divided into groups based on ocular symptoms reported at initial visit. This division was based on the OSDI cut-off value recommended by DEWS II in its *Diagnostic Methodology* report[25]. Subjects were divided into asymptomatic and symptomatic group, with OSDI of 13 considered as the threshold between no DED and mild DED symptoms. OSDI mean values, medians, ranges and standard deviations resulted from this division are shown in Table 15.

Table 15. OSDI reported over the time-course of the study in subjects divided into groups based on their OSDI score reported at initial visit

OSDI in subjects initially reported as **asymptomatic** (OSDI < 13)						
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	4.9 ± 4.0	5.6 ± 4.5	7.5 ± 8.0	10.3 ± 8.5	11.4 ± 13.2	2.2 ± 3.7
Median	4.6	4.4	5.2	8.3	8.3	0.0
Range	[0, 10]	[0, 15]	[0, 35]	[0, 38]	[0, 59]	[0, 13]
OSDI in subjects initially reported as **symptomatic (**OSDI ≥ 13)						
Mean ± SD	23.3 ± 10.1	16.6 ± 16.0	15.3 ± 11.8	13.6 ± 9.6	13.6 ± 9.8	10.2 ± 12.1
Median	21	11	13	9	11	4
Range	[13, 48]	[0, 61]	[0, 52]	[2, 35]	[2, 40]	[0, 38]

N – number of subjects; M – number of novices; SD – standard deviation; OSDI – Ocular Surface Disease Index; Asymptomatic < 13; Mild 13-22; Moderate 23-32; Severe ≥ 33 [25];

For initially asymptomatic subjects, statistically significant changes in OSDI were observed over the time-course of the study ($\chi^2 = 20.89$, $P < 0.001$). Post-hoc comparison between each pair of visits showed statistically significant differences between Baseline and 6-month ($P = 0.002$), Baseline and 12-month ($P = 0.011$), 2-week and 6-month ($P = 0.002$), 2-week and 12-month ($P = 0.010$), 3-month and 6-month ($P = 0.023$) and 3-month and 12-month visit ($P = 0.024$). Additionally, significant difference was noted in OSDI between Baseline and Control visit ($P = 0.018$).

For initially symptomatic subjects, statistically significant downward trend in OSDI was observed over the time-course of the study ($\chi^2 = 18.28$, $P = 0.001$), particularly between Baseline and any other visit ($P = 0.004$, $P = 0.005$, $P < 0.001$, $P = 0.002$ for 2-week, 3-month, 6-month and 12-month, respectively). Additionally, significant difference was noted between Baseline and Control visit ($P < 0.001$).

3.3.4. Reported symptoms - DEQ-5

The group mean and median values, standard deviations and ranges of DEQ-5 reported over the time-course of the study were shown in Table 16. There were no statistically significant differences in DEQ-5 since Baseline to 12-month visit ($\chi^2 = 7.31$, $P = 0.121$), however Wilcoxon test showed statistically significant difference in DEQ-5 between Baseline and Control visit ($P = 0.022$).

Table 16. DEQ-5 questionnaire scores reported over the time-course of the study

DEQ- 5 score [-]						
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	8 ± 6	6 ± 4	7 ± 4	7 ± 4	6 ± 4	5 ± 4
Median	7	6	6	6	6	6
Range	[0, 22]	[0, 15]	[0, 17]	[0, 21]	[0, 19]	[0, 12]

SD - standard deviation; DEQ-5 score > 5 - dry eye; DEQ-5 score > 12 - severe dry eye symptoms[25]

Subsequently, subjects were divided into groups based on ocular symptoms reported with DEQ-5 at initial visit[25]. Thus, subjects were divided into asymptomatic and symptomatic group, with DEQ-5 of 6 [-] being the threshold between DED and non-DED. DEQ-5 scores resulting from this division were displayed in Table 17.

Table 17. DEQ-5 scores reported over the time-course of the study in subjects divided into groups based on their initial DEQ-5 score

DEQ-5 in subjects initially reported as **asymptomatic** (DEQ-5 < 6)						
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	2 ± 2	5 ± 3	6 ± 3	6 ± 5	7 ± 5	5 ± 4
Median	2	5	6	6	7	4
Range	[0, 5]	[0, 12]	[0, 13]	[0, 21]	[0, 19]	[0, 12]
DEQ-5 in subjects initially reported as **symptomatic (**OSDI ≥ 6)						
Mean ± SD	11 ± 4	7 ± 4	7 ± 4.	7 ± 4	6 ± 3	5 ± 3
Median	11	6	6.	6	6	4
Range	[6, 22]	[0, 15]	[0, 17]	[0, 19]	[0, 12]	[0, 12]

DEQ-5 score > 5 - dry eye, DEQ-5 score > 12 - severe dry eye symptoms [25]

For asymptomatic subjects, statistically significant differences in DEQ-5 were recorded (χ^2 = 23.21, $P < 0.001$). Post-hoc analysis showed statistically significant differences in DEQ-5 between Baseline and all other visits ($P < 0.001$, $P < 0.001$, $P < 0.001$ and $P = 0.001$, for 2-week, 3-month, 6-month and 12-month, respectively). No statistically significant difference was noted between Baseline and Control visit ($P = 0.064$).

Also, for the symptomatic group changes were observed since Baseline until 12-month visit ($\chi^2 = 42.49$, $P < 0.001$), with a significant drop of DEQ-5 noted between Baseline and any other visit ($P < 0.001$, $P < 0.001$, $P < 0.001$ and $P < 0.001$, for 2-week, 3-month, 6-month and 12-month, respectively). Additionally, statistically significant difference was noted between Baseline and Control visit ($P < 0.001$).

3.3.5. Tear osmolarity

Sample size n, for which the difference of 2 mOsm/L in tear osmolarity could be discriminated at 5% level of significance and 90% power was estimated to be at $n \geq 50$. The group mean and median values, standard deviations and ranges of tear osmolarity over the time-course of the study are shown in Table 18.

Table 18. Tear osmolarity measures reported over the time-course of the study

Tear osmolarity [mOsm/L]					
OD					
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	304 ± 9	300 ± 9	297 ± 7	296 ± 8	293 ± 8
Median	302	299	297	296	293
Range	[289, 333]	[284, 330]	[284, 325]	[282, 313]	[281, 314]
OS					
Mean ± SD	302 ± 7	299 ± 9	296 ± 8	296 ± 7	292 ± 9
Median	300	298	297	296	291
Range	[291, 330]	[283, 321]	[282, 317]	[282, 310]	[279, 315]
Difference between OD and OS	***P* = 0.576**	***P* = 0.723**	***P* = 0.592**	***P* = 0.867**	***P* = 0.579**

OD - right eye; OS - left eye; SD- Standard deviation, * denotes statistical significance

Statistically significant differences in tear osmolarity over the time-course of the study were noted for right ($P < 0.001$, $\chi^2 = 22.91$) and left eye ($P = 0.001$, $\chi^2 = 15.54$). Post-hoc analysis showed statistically significant differences in right eye tear osmolarity between Baseline and 3-month, Baseline and 6-month, Baseline and 12-month and 3-month and 12-month visit ($P = 0.024$, $P < 0.001$, $P < 0.001$ and $P = 0.003$, respectively) and for the left eye tear osmolarity between Baseline and 3-month, Baseline and 6-month, Baseline and 12-month and 3-month and 12-month visit ($P = 0.034$, $P = 0.002$, $P < 0.001$, $P = 0.009$, respectively).

Evident downward trend of tear osmolarity was observed over the time-course of the study, even after subject refraining from wearing contact lenses for three days. Additionally, Wilcoxon test showed statistically significant differences in right eye ($P < 0.001$) and left eye ($P < 0.001$) tear osmolarity between Baseline and Control visit. Further investigation of the data revealed that subjects who initially reported increased osmolarity (hyperosmolarity, specifically ≥ 308 mOsm/L for at least one eye) exhibited its most apparent decrease. This decrease was observed as a steady decay in tear osmolarity for up to six months after the Baseline visit.

Statistically significant differences in tear osmolarity for subject reported with initially hyperosmotic tears were noted over the time-course of the study for right ($\chi^2 = 27.59$, $P < 0.001$) and left eye ($\chi^2 = 25.83$, $P < 0.001$) and particularly for the right eye between Baseline and other visits ($P = 0.005$, $P < 0.001$, $P < 0.001$ for 3-month, 6-month and 12-month visit, respectively) and between 3-month and 6-month visit ($P = 0.005$) and for the left eye between Baseline and other visits ($P = 0.002$, $P = 0.001$, $P < 0.001$ for 3-month, 6-month and 12-month visit, respectively) and between 3-month and 6-month visit ($P = 0.028$). Table 19 shows tear osmolarity measures assessed over the time-course of the study with respect to initially reported or not reported hyperosmolarity (≥ 308 mOsm\L) in at least one eye.

Table 19. Tear osmolarity values over the time-course of the study for two groups of subjects

Tear osmolarity in subjects with **initially hyperosmotic tears**					
OD					
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	316 ± 6	306 ± 10	297 ± 5	299 ± 7	290 ± 8
Median	316	305	296	299	288
Range	[309, 333]	[292, 330]	[291, 308]	[289, 313]	[282, 306]
OS					
Mean ± SD	312 ± 6	303 ± 9	297 ± 10	296 ± 5	292 ± 8
Median	311	302	297	295	292
Range	[306, 330]	[286, 321]	[282, 317]	[288, 304]	[279, 305]
Tear osmolarity in subjects with **initially normal tear osmolarity**					
OD					
Mean ± SD	299 ± 5	298 ± 9	297 ± 8	295 ± 8	294 ± 7
Median	299	298	298	294	294
Range	[289, 307]	[284, 317]	[284, 325]	[282, 311]	[281, 314]
OS					
Mean ± SD	299 ± 5	298 ± 9	297 ± 8	296 ± 8	292 ± 7
Median	299	297	297	296	291
Range	[291, 305]	[283, 320]	[285, 315]	[282, 310]	[279, 315]

N – number of subjects; M – number of novices; OD – right eye; OS – left eye; SD – standard deviation; high osmolarity in at least one eye ≥ 308 mOsm/L corresponds to the threshold between healthy eyes and mild DED symptoms

In subjects initially presented with lower tear osmolarity (< 308 mOsm/L) the decrease in tear osmolarity was also observed, however it was not reported to be statistically significant ($\chi^2 = 6.86$, $P = 0.077$ and $\chi^2 = 2.58$, $P = 0.461$ for left and right eye, respectively). The tear osmolarity readings for these subjects over the time-course of the study were within the range reported for healthy non-wearers. Singular subjects reported a temporary increase in tear osmolarity over the threshold of 308 mOsm/L, however these

values were not accompanied by any other signs of DED used as screening criteria in the study, as defined in the previous sub-section of this chapter.

No statistically significant difference was observed in the inter-eye difference in tear osmolarity over the time-course of the study ($\chi^2 = 3.11$, $P = 0.375$). Wilcoxon test showed no statistically significant difference between Baseline and Control visit ($P = 0.971$) in this ocular measure.

3.3.6. Non-invasive Keratograph tear film break-up time

Table 20. Reported M-NIKBUT values measured with K5M

	M-NIKBUT [s]					
	OD					
Visit	**Baseline (PC)**	**Day 2 (PL)**	**3-month (PL)**	**6-month (PL)**	**12-month (PL)**	**Control (PC)**
Mean ± SD	17.6 ± 4.3	15.4 ± 3.0	14.6 ± 2.6	14.4 ± 3.1	14.7 ± 3.7	15.0 ± 5.2
Median	18.3	15.2	14.7	14.6	14.9	15.9
Range	[8.8, 24.9]	[11.2, 24.5]	[4.9, 20.0]	[8.6, 21.5]	[7.2, 23.1]	[7.4, 24.9]
	OS					
Mean ± SD	16.9 ± 5.1	14.9 ± 3.2	16.1 ± 2.6	14.9 ± 3.1	15.6 ± 3.6	14.7 ± 4.9
Median	17.3	15.1	15.6	15.5	15.5	12.9
Range	[6.5, 24.9]	[3.5, 22.7]	[11.5, 22.9]	[5.8, 20.1]	[3.6, 23.7]	[8.9, 24.9]
Difference between OD and OS	***P* =0.469**	***P* =0.605**	***P* = 0.012***	***P* = 0.254**	***P* = 0.416**	**-**

*M-NIKBUT - Mean non-invasive keratography tear film break-up time; PC – pre-corneal tear film; PL – pre-lens tear film; * denotes statistical significance*

Statistically significant differences in right eye M-NIKBUT were noted since Baseline until 12-month visit ($\chi^2 = 23.72$, $P < 0.001$) particularly between Baseline and other visits ($P = 0.004$, $P < 0.001$, $P < 0.001$, $P < 0.001$ for Day 2, 3-month, 6-month and 12-month

visit, respectively). These differences were not statistically significant for the left eye (χ^2 = 6.54, P = 0.162). Corresponding M-NIKBUT measures are shown in Table 20. Additionally, Wilcoxon test showed statistically significant difference in M-NIKBUT between Baseline and Control visit for right eye (P = 0.048), however not for the left eye (P = 0.187).

Table 21. F-NIKBUT values measured with K5M reported over the time-course of the study

F-NIKBUT [s]						
OD						
Visit	**Baseline (PC)**	**Day 2 (PL)**	**3-month (PL)**	**6-month (PL)**	**12-month (PL)**	**Control (PC)**
Mean ± SD	14.8 ± 5.4	9.6 ± 4.1	7.9 ± 3.3	8.7 ± 4.5	8.9 ± 4.3	12.4 ± 5.44
Median	14.5	8.7	7.5	7.7	7.5	12.3
Range	[2.5, 24.9]	[4.5, 24.5]	[2.1, 16.5]	[2.4, 24.0]	[3.1, 22.9]	[5.6, 24.9]
OS						
Mean ± SD	14.0 ± 6.3	8.5 ± 3.9	9.4 ± 4.2	8.4 ± 3.8	9.1 ± 4.8	12.2 ± 6.3
Median	12.3	7.7	8.4	7.3	7.9	10.2
Range	[3.3, 24.9]	[2.3, 20.1]	[3.8, 21.9]	[3.9, 19.7]	[3.51, 23.5]	[3.5, 24.9]
Difference between OD and OS	***P* = 0.331**	***P* = 0.181**	***P* = 0.050**	***P* = 0.839**	***P* = 0.862**	**-**

*PC – pre-corneal tear film; PL – pre-lens tear film; * denotes statistical significance; OS - left eye*

Statistically significant differences were noted for right eye F-NIKBUT (χ2 = 55.52, P < 0.001), particularly between Baseline and other visits (P < 0.001 for Day 2, 3-month, 6-month and 12-month visit, respectively). For the left eye the same statistical dependence was noted (χ^2 = 41.12, P < 0.001), with statistically significant differences noted particularly between Baseline and other visits (P < 0.001 for Day 2, 3-month, 6-month and 12-month visit, respectively). Additionally, no statistically significant difference in F-NIKBUT was noted between Baseline and Control visit for the right eye (P = 0.074) and

left eye (P = 0.289). F-NIKBUT values reported over the time-course of the study are shown in Table 21. Linear correlations between different measures of tear stability assessed in the study, including FBUT, F-NIKBUT and M-NIKBUT are shown in Table 22.

Table 22. Linear correlations reported between different measures of tear film stability reported in the longitudinal study

Ocular measure	**M-NIKBUT OS**	**F-NIKBUT OD**	**F-NIKBUT OS**	**FBUT OD**	**FBUT OS**
M-NIKBUT OD	***R* = 0.294** ***P* = 0.001***	***R* = 0.522** ***P* < 0.001***	***R* = 0.347** ***P* < 0.001***	***R* = 0.276** ***P* = 0.001***	***R* = 0.334** ***P* < 0.001***
M-NIKBUT OS		***R* = 0.292** ***P* = 0.001***	***R* = 0.818** ***P* < 0.001***	***R* = 0.329** ***P* < 0.001***	***R* = 0.360** ***P* < 0.001***
F-NIKBUT OD			***R* = 0.501** ***P* < 0.001***	***R* = 0.250** ***P* = 0.004***	***R* = 0.333** ***P* < 0.001***
F-NIKBUT OS				***R* = 0.403** ***P* < 0.001***	***R* = 0.464** ***P* < 0.001***
FBUT OD					***R* = 0.863** ***P* < 0.001***

OD - right eye, OS - left eye, M-NIKBUT - mean non-invasive keratography tear film break-up time, F-NIKBUT - first non-invasive keratography tear film break-up time, FBUT - fluorescein tear film break-up time

3.3.7. Meniscometry

Table 23 shows TMH values assessed with K5M-based *en face* method over the time-course of the study. Friedman test showed no statistically significant differences in lower central TMH measured with Oculus K5M since Baseline until 12-month visit, for neither right ($\chi^2 = 0.84$, $P = 0.839$), nor the left eye ($\chi^2 = 4.12$, $P = 0.249$). Additionally, Wilcoxon test showed no statistically significant difference between Baseline and Control visit for right ($P = 0.139$) and left eye ($P = 0.220$).

Table 23. Inferior central tear meniscus height measured with K5M

	TMH_{KFM} [mm]				
	OD				
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	0.25 ± 0.06	0.24 ± 0.07	0.24 ± 0.07	0.25 ± 0.07	0.23 ± 0.07
Median	0.24	0.24	0.24	0.23	0.23
Range	[0.13, 0.36]	[0.15, 0.58]	[0.14, 0.48]	[0.12, 0.43]	[0.16, 0.39]
	OS				
Mean ± SD	0.25 ± 0.07	0.24 ± 0.06	0.25 ± 0.07	0.24 ± 0.06	0.23 ± 0.06
Median	0.25	0.22	0.24	0.23	0.23
Range	[0.15, 0.46]	[0.13, 0.44]	[0.14, 0.52]	[0.15, 0.38]	[0.12, 0.36]
Difference between OD and OS	***P* = 0.940**	***P* = 0.978**	***P* = 0.760**	***P* = 0.742**	**-**

TMH_{KFM} - Keratograph 5M-based tear meniscus height; OD - right eye, OS-left eye; SD-standard deviation

Tables below show group mean, median values, ranges and standard deviation of different geometrical parameters of tear meniscus assessed with dynamic OCT method, as presented in the ***Experiment 3***. D-TMH values are shown in Table 24, D-TMD in Table 25 and D-TMA in Table 26.

Table 24. Tear meniscus height based on OCT B-scan sequence, reported in the study

D-TMH [mm] OS						
Visit	**Baseline**	**2-weeks**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	0.27 ± 0.12	0.24 ± 0.14	0.20 ± 0.12	0.22 ± 0.12	0.20 ± 0.11	0.21 ± 0.11
Median	0.26	0.23	0.17	0.20	0.17	0.22
Range	[0.05, 0.54]	[0.02, 0.59]	[0.01, 0.50]	[0.01, 0.47]	[0.01, 0.47]	[0.04, 0.43]

SD - standard deviation, D-TMH - tear meniscus height based on dynamic meniscometry; OS - left eye

Table 25. Tear meniscus depth based on OCT B-scan sequence, reported in the study

D-TMD [mm] OS						
Visit	**Baseline**	**2-weeks**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	0.16 ± 0.02	0.15 ± 0.02	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.03	0.17 ± 0.03
Median	0.16	0.15	0.16	0.16	0.16	0.16
Range	[0.11, 0.20]	[0.09, 0.21]	[0.09, 0.20]	[0.09, 0.21]	[0.09, 0.23]	[0.12, 0.23]

SD - standard deviation, D-TMD - tear meniscus depth-based on dynamic meniscometry; OS - left eye

Table 26. Tear meniscus area based on OCT B-scan sequence, reported in the study

D-TMA [mm^2] OS						
Visit	**Baseline**	**2-weeks**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	0.012 ± 0.004	0.012 ± 0.004	0.013 ± 0.004	0.013 ± 0.004	0.014 ± 0.005	0.015 ± 0.005
Median	0.012	0.013	0.014	0.013	0.015	0.015
Range	[0.004, 0.023]	[0.004, 0.021]	[0.003, 0.020]	[0.002, 0.020]	[0.003, 0.023]	[0.004, 0.023]

SD - standard deviation, TMA - tear meniscus area; OS - left eye

Statistically significant differences were noted in D-TMH (χ^2 = 12.86, P = 0.012), particularly between Baseline and 3-month (P = 0.003), Baseline and 12-month (P = 0.008), 2-week and 3-month (P = 0.085) and 2-week and 12-month (P = 0.077).

Additionally, statistically significant difference was noted between Baseline and Control visit ($P = 0.031$).

Statistically significant differences were not noted in D-TMD ($\chi^2 = 5.40$, $P = 0.246$) since Baseline until 12-month visit and between Baseline and Control visit ($P = 0.218$).

Statistically significant differences were noted in D-TMA ($\chi^2 = 12.56$, $P = 0.014$), particularly between Baseline and 12-month ($P = 0.003$), 3-month and 12-month ($P = 0.023$) and 6-month and 12-month ($P = 0.022$). Statistically significant difference was noted in D-TMA between Baseline and Control visit ($P = 0.010$).

3.3.8. Tear Clearance Rate

Mean, median values, ranges and standard deviations of TCR_{TMH} (Table 27), TCR_{TMD} (Table 28) and TCR_{TMA} (Table 29) are shown below. Corresponding tear meniscus dynamics based on which the TCR was calculated is shown in Figure 35.

Table 27. TMH-based tear clearance rates reported over the time-course of the study

	TCR_{TMH} [%/30s]				
	OS				
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	21.6 ± 20.1	20.1 ± 24.8	15.7 ± 15.4	15.8 ± 22.2	22.0 ± 15.1
Median	20.9	24.5	18.7	18.6	18.8
Range	[-13.9, 73.8]	[-55.9, 83.7]	[-15.1, 42.7]	[-57.6, 57.7]	[1.9, 59.3]

SD- standard deviation; OS - left eye; TCR - tear clearance rate based on TMH - tear meniscus height

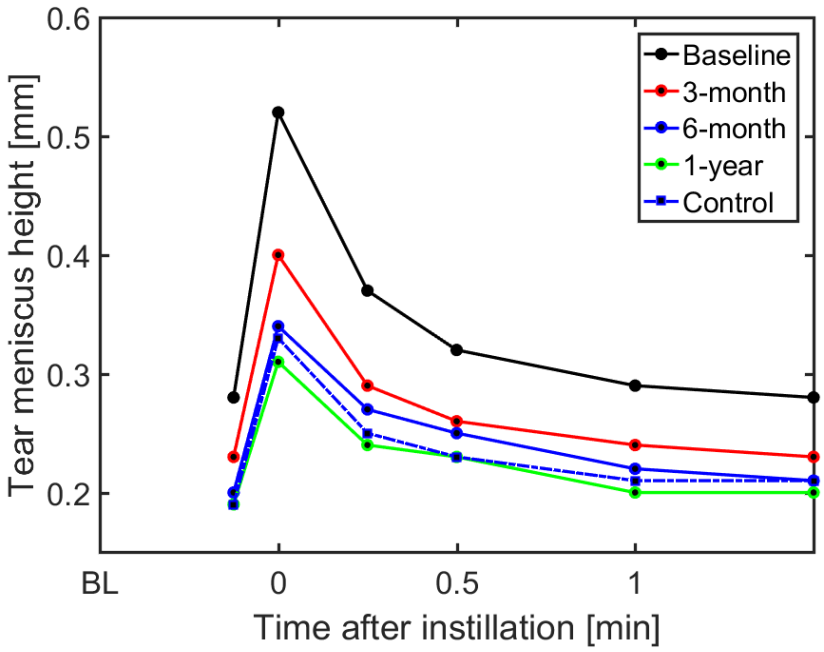


Figure 35. Reported dynamic changes of tear meniscus height after saline instillation

Statistically significant differences were noted in TCR_{TMH} (χ^2 = 9.95, P = 0.019), particularly between Baseline and 12-month (P = 0.030). Significant difference was not noted in TCR_{TMH} between Baseline and Control visit (P = 0.828).

Table 28. TMD-based tear clearance rates reported over the time-course of the study

	TCR_{TMD} [%/30s] OS				
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	18.0 ± 18.4	15.1 ± 19.1	12.1 ± 15.7	8.3 ± 20.6	18.6 ± 15.4
Median	19.0	16.8	12.4	11.8	14.5
Range	[-29.4, 68.6]	[-45.8, 74.6]	[-19.1, 41.9]	[-79.1, 34.5]	[1.3, 56.7]

TCR - tear clearance rate based on: TMD - tear meniscus depth; SD- standard deviation; OS - left eye

Statistically significant differences were noted in TCR_{TMD} (χ^2 = 8.92, P = 0.030), particularly between Baseline and 12-month (P = 0.005). Statistically significant difference was not noted between Baseline and Control visit (P = 0.785).

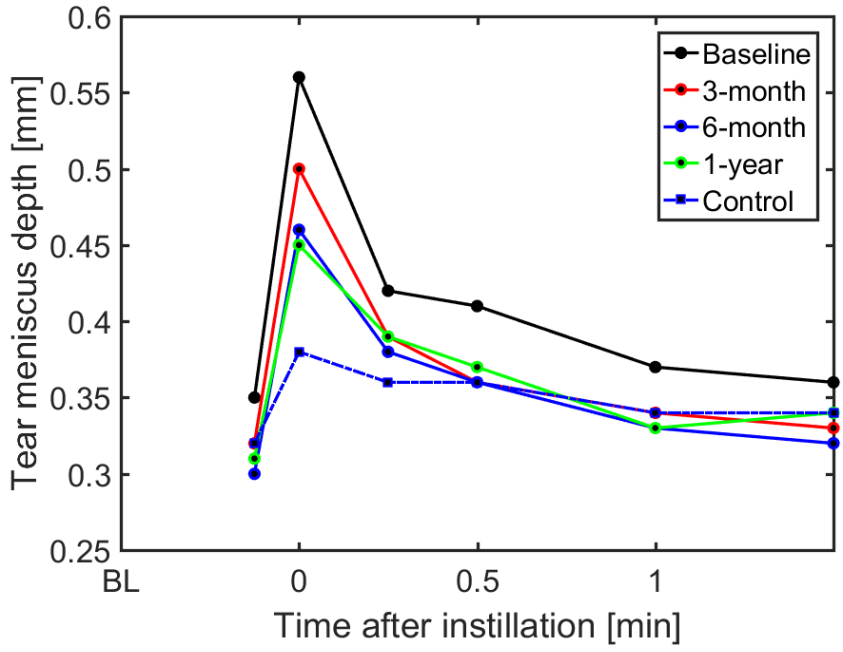


Figure 36. Mean reported dynamic changes of tear meniscus depth after saline instillation

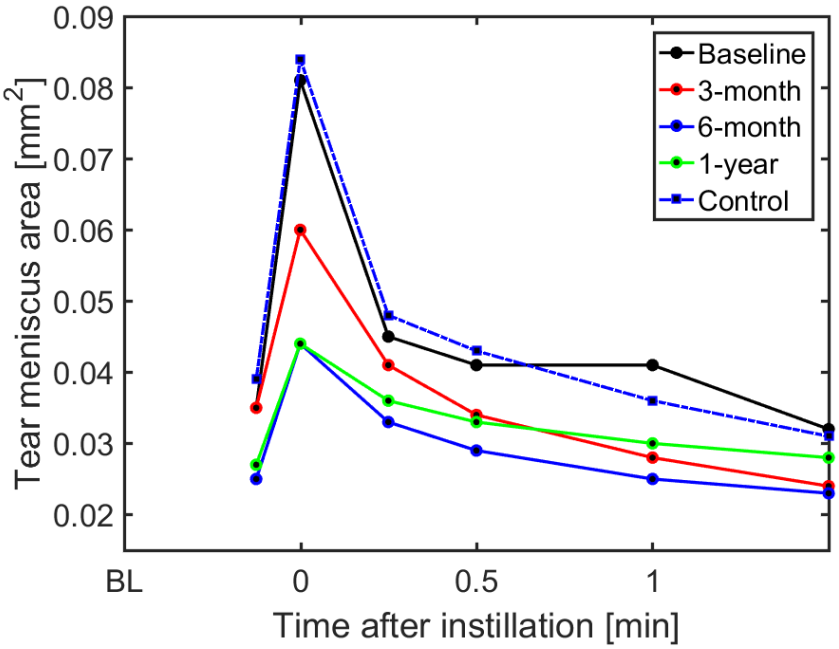


Figure 37. Mean reported dynamic changes of tear meniscus area after saline instillation

Statistically significant differences were noted in TCR_{TMA} (χ^2 = 9.95, P = 0.019), particularly between Baseline and 12-month (P = 0.005). Statistically significant difference was not noted in TCR_{TMA} between Baseline and Control visit (P = 0.751).

Table 29. TMA-based tear clearance rates reported over the time-course of the study

TCR_{TMA} [%/30s] OS					
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	28.8 ± 31.1	24.3 ± 35.7	24.0 ± 21.0	19.8 ± 24.0	35.1 ± 20.3
Median	31.0	31.3	23.2	22.3	29.1
Range	[-88.2, 95.1]	[-87.9, 93.6]	[-18.6 56.8]	[-51.5, 61.2]	[6.1, 82.4]

TCR - tear clearance rate based on: TMD - tear meniscus depth; SD- standard deviation; OS - left eye

3.3.9. Bulbar and limbal redness

Mean and median values, ranges and standard deviation of bulbar and limbal ocular redness are shown in Table 30.

Table 30. Bulbar and limbal redness scores reported over the time-course of the study

	Baseline	3-month	6-month	12-month	Control
Bulbar redness [-]					
OD					
Mean ± SD	0.77 ± 0.31	0.73 ± 0.32	0.73 ± 0.29	0.72 ± 0.35	0.74 ± 0.31
Median	0.75	0.65	0.65	0.60	0.68
Range	[0.15, 1.75]	[0.20, 1.65]	[0.30, 1.45]	[0.20, 1.80]	[0.25, 1.30]
OS					
Mean ± SD	0.77 ± 0.27	0.79 ± 0.35	0.76 ± 0.33	0.72 ± 0.32	0.75 ± 0.26
Median	0.70	0.75	0.70	0.65	0.80
Range	[0.30, 1.45]	[0.25, 1.75]	[0.30, 1.70]	[0.25, 1.85]	[0.45, 1.30]
Difference between OD and OS	***P* = 0.924**	***P* = 0.375**	***P* = 0.679**	***P* = 0.651**	***P* = 0.300**
Limbal redness [-]					
OD					
Mean ± SD	0.53 ± 0.27	0.51 ± 0.27	0.54 ± 0.29	0.55 ± 0.33	0.52 ± 0.20
Median	0.50	0.50	0.45	0.45	0.53
Range	[0.20, 1.35]	[0.10, 1.15]	[0.10, 1.40]	[0.05, 1.40]	[0.15, 0.90]
OS					
Mean ± SD	0.52 ± 0.25	0.52 ± 0.28	0.53 ± 0.28	0.48 ± 0.26	0.49 ± 0.25
Median	0.50	0.45	0.50	0.45	0.55
Range	[0.10, 1.10]	[0.10, 1.35]	[0.10, 1.50]	[0.03, 1.30]	[0.00, 0.85]
Difference between OD and OS	***P* = 0.758**	***P* = 0.710**	***P* = 0.993**	***P* = 0.565**	***P* = 0.879**

*SD – Standard deviation, * denotes statistical significance; OD - right eye; OS - left eye*

No statistically significant differences over the time-course of the study were noted in bulbar for neither right ($\chi^2 = 2.36$, $P = 0.501$) nor for the left eye ($\chi^2 = 2.69$, $P = 0.441$) and

in limbal redness for neither right (χ^2 = 2.40, P = 0.413) nor for the left eye (χ^2 = 4.25, P = 0.236). Additionally, no statistically significant differences were noted with Wilcoxon test between Baseline and Control visit in bulbar redness score (P = 0.668, P = 0.863 for right and left eye, respectively) and in limbal redness score (P = 0.594, P = 0.950 for right and left eye, respectively).

3.3.10. Ocular staining scores with vital dyes - conjunctiva

Mean and median values, ranges and standard deviation of conjunctival fluorescein staining score reported in the time-course of the study are shown in Table 31.

Table 31. Conjunctival fluorescein staining scores reported over the time-course of the study

Conjunctival staining score [-]						
OD						
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	0.8 ± 0.5	0.8 ± 0.4	0.9 ± 0.5	1.0 ± 0.4	1.0 ± 0.6	0.7 ± 0.5
Median	1.0	1.0	1.0	1.0	1.0	1.0
Range	[0.0, 1.5]	[0.0 1.5]	[0.0, 2.5]	[0.0. 2.0]	[0.0, 2.0]	[0.0, 1.5]
OS						
Mean ± SD	0.8 ± 0.5	0.8 ± 0.4	0.9 ± 0.5	1.0 ± 0.4	1.0 ± 0.5	0.6 ± 0.6
Median	1.0	1.0	1.0	1.0	1.0	1.0
Range	[0.0, 2.5]	[0.0, 1.5]	[0.0, 2.5]	[0.0, 2.0]	[0.0, 2.0]	[0.0, 1.5]
Difference Between OD and OS	***P* = 0.736**	***P* = 0.429**	***P* = 0.753**	***P* = 0.760**	***P* = 0.714**	**-**

SD- standard deviation; OD - right eye, OS - left eye

Statistically significant changes were observed in conjunctival staining score over the time-course of the study, for right eye only (χ^2 = 12.32, P = 0.015), but not for the left eye (χ^2 = 7.12, P = 0.130). Post-hoc analysis showed statistically significant differences in

conjunctival staining between Baseline and 6-month (P = 0.023), Baseline and 12-month (P = 0.033) and 2-week and 6-month visit (P = 0.013) for the right eye. Additionally, Wilcoxon test showed no statistically significant difference in conjunctival staining between Baseline and Control visit for neither right (P = 0.636), nor for the left eye (P = 0.272).

3.3.10. Ocular staining scores with vital dyes - cornea

Mean and median values, ranges and standard deviation of corneal fluorescein staining score reported in the time-course of the study are shown in Table 32.

Table 32. Corneal fluorescein staining scores reported in the study

Corneal staining score [-]						
OD						
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	0.5 ± 0.3	0.4 ± 0.4	0.3 ± 0.3	0.3 ± 0.3	0.2 ± 0.3	0.1 ± 0.2
Median	0.5	0.5	0.0	0.5	0.0	0.0
Range	[0.0, 1.5]	[0.0, 1.5]	[0.0, 1.0]	[0.0, 1.0]	[0.0, 1.0]	[0.0, 0.5]
OS						
Mean ± SD	0.5 ± 0.5	0.4 ± 0.4	0.3 ± 0.3	0.4 ± 0.3	0.3 ± 0.4	0.1 ± 0.2
Median	0.5	0.0	0.0	0.5	0.0	0.0
Range	[0.0, 2.5]	[0.0, 1.5]	[0.0, 1.0]	[0.0, 1.0]	[0.0, 1.5]	[0.0, 0.5]
Difference Between OD and OS	***P* = 0.313**	***P* = 0.744**	***P* = 0.989**	***P* = 0.509**	***P* = 0.240**	**-**

SD- standard deviation; OD - right eye, OS - left eye

Statistically significant changes were observed in the right eye corneal staining score over the time-course of the study for right (χ^2 = 23.66, P < 0.001), however not for the left eye (χ^2 = 9.29, P = 0.054). Post-hoc analysis showed statistically significant differences in right

eye corneal staining score between Baseline and 3-month (P = 0.001), Baseline and 6-month (P = 0.032), Baseline and 12-month (P < 0.001) and 6-month and 12-month visit (P = 0.028). Additionally, statistically significant changes were noted between Baseline and Control visit in corneal staining score for the right eye (P < 0.001) and for the left eye (P = 0.018).

3.3.11. Lid wiper epitheliopathy score

Statistically significant changes were noted in LWE scores over the time-course of the study. Reported values of LWE are summarized in Table 33. Changes were noted for the right eye upper LWE score ($\chi 2$ = 20.94, P < 0.001), particularly between 3-month and 12-month (P = 0.001) and 6-month and 12-month visit (P < 0.001), for the right eye lower LWE score (χ^2 = 29.79, P < 0.001) between Baseline and 12-month (P = 0.002), 3-month and 6-month (P = 0.032), 3-month and 12-month (P < 0.001), 6-month and 12-month (P < 0.001), for the left eye LWE score (χ^2 = 13.57, P = 0.004), particularly between 3-month and 12-month visit (P = 0.001) and 6-month and 12-month visit (P < 0.001) and for the left eye lower LWE score (χ^2 = 31.50, P < 0.001), particularly between 3-month and 6-month visit (P < 0.001), 3-month and 12-month (P < 0.001) and 6-month and 12-month visit (P < 0.001).

Table 33. Upper and lower lid wiper epitheliopathy scores reported in the study

Upper LWE score [-]					
			OD		
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	0.7 ± 1.0	0.5 ± 0.7	0.4 ± 0.5	0.9 ± 0.6	0.8 ± 0.7
Median	0.0	0.0	0.0	1.0	1.0
Range	[0.0, 3.0]	[0.0, 2.0]	[0.0, 2.0]	[0.0, 3.0]	[0.0, 2.0]
			OS		
Mean ± SD	0.7 ± 0.9	0.4 ± 0.7	0.4 ± 0.5	0.8 ± 0.7	0.8 ± 0.5
Median	0.0	0.0	0.0	1.0	1.0
Range	[0.0, 3.0]	[0.0, 3.0]	[0.0, 2.0]	[0.0, 3.0]	[0.0, 1.0]
Difference between OD and OS	***P* = 0.544**	***P* = 0.665**	***P* = 0.705**	***P* = 0.443**	**-**
Lower LWE score [-]					
			OD		
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	0.7 ± 0.7	0.7 ± 0.7	0.5 ± 0.6	1.1 ± 0.6	1.1 ± 0.6
Median	1.0	1.0	0.0	1.0	1.0
Range	[1.0, 3.0]	[0.0, 3.0]	[0.0, 2.0]	[0.0, 3.0]	[0.0, 3.0]
			OS		
Mean ± SD	0.6 ± 0.7	0.7 ± 0.7	0.6 ± 0.6	1.1 ± 0.5	1.0 ± 0.6
Median	0.0	1.0	1.0	1.0	1.0
Range	[0.0, 2.0]	[0.0, 2.0]	[0.0, 2.0]	[0.0, 2.0]	[0.0, 2.0]
Difference between OD and OS	***P* = 0.556**	***P* = 0.763**	***P* = 0.292**	***P* = 0.542**	**-**

*SD – standard deviation, * denotes statistical significance; OD - right eye; OS - left eye*

Additionally, Wilcoxon test showed no statistically significant differences in LWE scores between Baseline and Control visit ($P = 0.518$, $P = 0.708$, $P = 0.666$ for right eye upper, left eye upper and right eye lower lid wiper, respectively), except for the lower left eyelid LWE score ($P = 0.048$).

3.3.12. Corneal thickness

Statistically significant changes in central corneal thickness (CCT) were found over the time-course of the study (χ^2 = 13.33, *P* = 0.010), particularly between Baseline and 12-month (*P* = 0.005), 2-week and 12-month visit (*P* = 0.002) and 3-month and 12-month visit (*P* = 0.003). Additionally, no statistically significant difference was noted between Baseline and Control visit (*P* = 0.173). CCT values reported over the time-course of the study are shown in Table 34.

Table 34. Central corneal thickness reported over the time-course of the study

	CCT [µm] OS					
Visit	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	571 ± 35	571 ± 34	571 ± 34	568 ± 34	569 ± 33	557 ± 34
Median	574	573	575	573	573	560
Range	[492, 640]	[496, 635]	[493, 633]	[492, 637]	[492, 630]	[494, 616]

SD – standard deviation; CCT - central corneal thickness; OS - left eye

3.3.13. Meibomian glands drop-out

Statistically significant differences were noted in the right upper lid Meibomian drop-out ($\chi2$ = 10.72, *P* = 0.013), particularly between Baseline and 3-month (*P* = 0.040), Baseline and 6-month (*P* = 0.034), 3-month and 12-month (*P* = 0.015) and 6-month and 12-month (*P* = 0.016) and for the left lower lid Meibomian gland drop-out ($\chi2$ = 10.57, *P* = 0.014), particularly between Baseline and 12-month (*P* = 0.017), 3-month and 12-month (*P* = 0.006) and 6-month and 12-month visit (*P* = 0.044). No statistically significant difference was noted in upper left ($\chi2$ = 5.81, *P* = 0.121) and lower right ($\chi2$ = 6.81, *P* = 0.121) Meibomian glands drop-out.

Additionally, Wilcoxon test showed no statistically significant difference between Meibo-scores of any of the eyelids ($P = 0.942$, $P = 0.800$, $P = 0.396$ and $P = 0.314$ for upper right, upper left, lower right and lower left, respectively) between Baseline and Control visit. Meibo-scores reported over the time-course of the study are shown in Table 35.

Table 35. Upper and lower eyelid meibography scores reported in the study

Upper eyelid Meibomian gland drop-out [%]					
		OD			
Visit	**Baseline**	**3-month**	**6-month**	**12-month**	**Control**
Mean ± SD	29 ± 15	27 ± 12	27 ± 12	31 ± 12	31 ± 11
Median	30	27	25	28	28
Range	[0, 63]	[0, 62]	[0, 60]	[0, 62]	[16, 65]
		OS			
Mean ± SD	30 ± 15	29 ± 12	30 ± 12	29 ± 10	35 ± 10
Median	29	27	29	28	30
Range	[0, 63]	[0, 58]	[0, 54]	[0, 55]	[22, 53]
Difference between OD and OS	***P* = 0.888**	***P* = 0.528**	***P* = 0.279**	***P* = 0.423**	-
Lower lid Meibomian gland drop-out [%]					
		OD			
Mean ± SD	34 ± 17	34 ± 15	32 ± 15	31 ± 14	32 ± 16
Median	36	33	32	32	33
Range	[0, 81]	[0, 81]	[0, 81]	[0, 72]	[0, 75]
		OS			
Mean ± SD	37 ± 17	37 ± 15	35 ± 15	31 ± 17	33 ± 18
Median	36	37	34	33	35
Range	[0, 85]	[0, 63]	[0, 63]	[0, 67]	[0, 68]
Difference between OD and OS	***P* = 0.379**	***P* = 0.134**	***P* = 0.202**	***P* = 0.811**	-

** denotes statistical significance; SD – standard deviation; OD - right eye; OS - left eye*

3.3.14. Lipid layer thickness

Figure 38 shows the percentage distribution of 3 different types of LLT scores assessed by a masked evaluator based on the recorded thin-layers interference patterns. Substantial increase in the quantity of 'thin' lipid layers was observed at 6-month visit and 12-month visit.

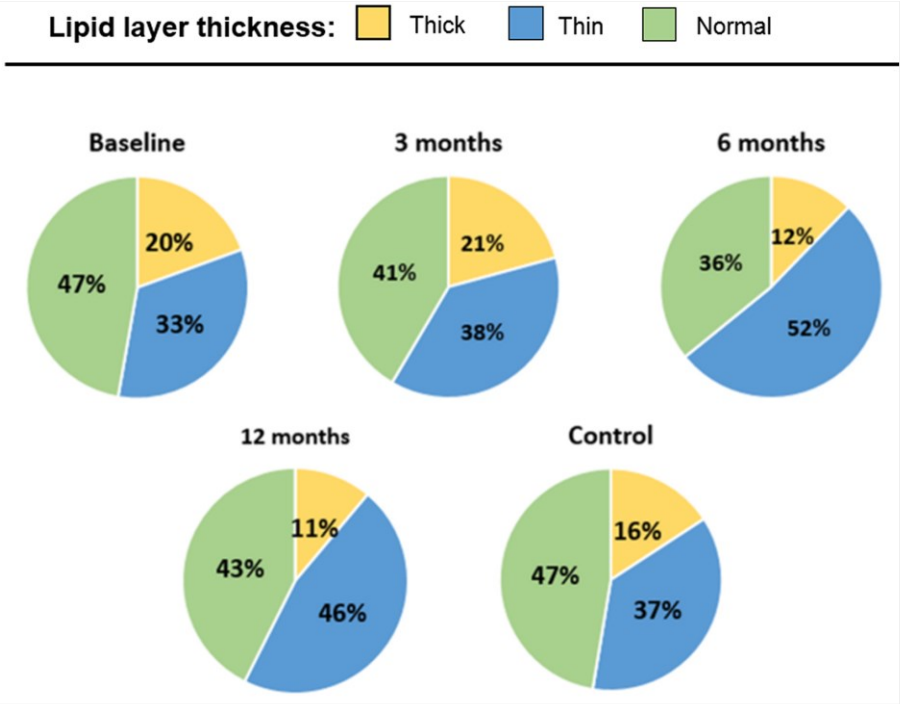


Figure 38. Reported proportions of 3 different types of lipid layers

Statistically significant differences were noted in LLT score over the time-course of the study ($\chi 2 = 14.94$, $P = 0.002$). Post-hoc analysis showed statistically significant differences between Baseline and 6-month ($P = 0.004$) and Baseline and 12-month ($P = 0.012$), 3-month and 6-month visit ($P = 0.005$) and 3-month and 12-month visit ($P = 0.030$). Statistically significant difference was not observed between Baseline and Control visit ($P = 0.713$).

3.3.15. Correlations between signs and symptoms

Tables 36-38 show the linear coefficient matrix between all the ocular measures assessed in the study and corresponding *P*-values. Coefficients are color-coded from the lowest negative (red) to highest positive value (green). *P*-values denoting statistically significant correlations ($P < 0.05$) were highlighted in light green.

Table 36. Linear correlation coefficients matrix between all the considered ocular measures reported in the longitudinal study and their corresponding P-values

P-value / R coefficient	Temperature	Relative humidity	OSDI	DEQ-5	TMHK5M	S-TMH	S-TMD	S-TMA	D-TMH	D-TMD
Temperature		0.285	0.997	0.708	0.619	0.822	0.842	0.538	0.894	0.660
Relative humidity	0.094		0.592	0.452	0.727	0.194	0.318	0.104	0.877	0.079
OSDI	0.000	-0.047		<.001	0.984	0.494	0.738	0.686	0.218	0.153
DEQ-5	0.033	-0.066	0.601		0.646	0.512	0.405	0.652	0.277	0.297
TMHK5M	-0.044	0.031	0.002	0.040		0.324	0.626	0.577	0.127	0.978
S-TMH	0.020	0.114	0.060	0.058	-0.087		<.001	<.001	0.693	<.001
S-TMD	0.017	0.088	0.029	0.073	-0.043	0.893		<.001	0.561	<.001
S-TMA	0.054	0.142	0.035	0.040	-0.049	0.879	0.937		0.491	<.001
D-TMH	0.012	-0.014	-0.108	-0.095	0.133	-0.035	-0.051	-0.061		<.001
D-TMD	-0.039	0.153	0.125	0.091	0.002	0.400	0.424	0.402	-0.465	
D-TMA	-0.017	0.065	0.037	0.045	-0.035	0.100	0.211	0.165	-0.617	0.707
Tear osmolarity	-0.030	-0.003	-0.046	0.015	0.018	-0.169	-0.131	-0.129	0.244	-0.066
M-NIKBUT	-0.018	0.130	0.111	0.053	-0.004	0.200	0.182	0.231	-0.096	0.114
F-NIKBUT	-0.021	0.114	0.101	0.070	0.006	0.170	0.190	0.227	-0.015	0.047
Bulbar redness	0.029	0.018	0.054	0.065	0.053	0.297	0.287	0.245	-0.041	0.119
Limbal redness	0.017	0.037	0.001	0.090	0.122	0.226	0.193	0.179	-0.090	0.106
FBUT	0.028	0.006	0.195	0.104	0.208	0.137	0.173	0.159	0.073	-0.009
Corneal staining	0.058	-0.080	0.064	0.025	0.023	-0.095	-0.087	-0.104	-0.118	-0.023
Conjunctival staining	-0.019	-0.008	-0.008	0.042	0.107	-0.010	0.033	-0.013	-0.152	0.062
CCT	0.031	0.026	0.049	0.072	0.206	0.121	0.042	0.105	0.059	-0.056
TCR_{TMH}	0.051	-0.034	0.011	-0.049	0.133	-0.173	-0.167	-0.096	0.030	-0.036
TCR_{TMD}	0.052	-0.034	-0.021	-0.015	0.119	-0.174	-0.190	-0.101	0.021	-0.076
TCR_{TMA}	0.075	-0.014	-0.048	-0.068	0.083	-0.213	-0.214	-0.125	-0.030	-0.117
Upper MG drop-out	0.088	-0.035	0.158	0.195	-0.024	0.037	0.059	0.071	-0.029	0.061
Lower MG drop-out	0.038	-0.102	-0.183	0.005	-0.100	0.165	0.255	0.215	-0.055	0.156
Upper LWE score	-0.102	0.252	-0.028	-0.056	0.076	-0.012	0.003	0.030	-0.021	0.102
Lower LWE score	-0.259	0.133	-0.155	-0.048	-0.082	-0.067	0.031	0.044	-0.167	0.078

OSDI - Ocular Surface Disease Index; TMH_{K5M} - K5M-based tear Meniscus height; S-TMH - OCT-based static tear meniscus height; S-TMD - OCT-based static tear meniscus depth; S-TMA- OCT based static tear meniscus area; D-TMH - OCT-based dynamic tear meniscus height; D-TMD - OCT-based dynamic tear meniscus depth; D-TMA - OCT based dynamic tear meniscus area; MG - Meibomian glands; FBUT - fluorescein tear film break-up time; CCT - central corneal thickness; LWE - lid wiper epitheliopathy score; TCR - tear clearance rate (TMH-based, TMD-based or TMA-based)

Table 37. Linear correlation coefficients matrix between all the considered ocular measures reported in the longitudinal study and their corresponding P-values

P-value / R coefficient	D-TMA	Tear osmolarity	M-NIKBUT	F-NIKBUT	Bulbar redness	Limbal redness	FBUT	Corneal staining	Conjunctival staining
Temperature	0.851	0.731	0.839	0.813	0.738	0.848	0.754	0.508	0.826
Relative humidity	0.457	0.968	0.138	0.195	0.840	0.671	0.947	0.362	0.932
OSDI	0.670	0.598	0.206	0.248	0.535	0.989	0.025	0.469	0.928
DEQ-5	0.608	0.868	0.547	0.424	0.456	0.304	0.236	0.773	0.635
TMHK5M	0.693	0.837	0.963	0.948	0.549	0.162	0.017	0.796	0.220
S-TMH	0.254	0.053	0.022	0.051	0.001	0.009	0.117	0.277	0.910
S-TMD	0.015	0.133	0.037	0.029	0.001	0.026	0.048	0.319	0.711
S-TMA	0.058	0.141	0.008	0.009	0.005	0.040	0.069	0.235	0.880
D-TMH	<.001	0.005	0.273	0.867	0.637	0.307	0.408	0.177	0.082
D-TMD	<.001	0.454	0.191	0.594	0.174	0.226	0.917	0.793	0.481
D-TMA		0.203	0.872	0.933	0.686	0.854	0.421	0.326	0.030
Tear osmolarity	-0.112		0.093	0.805	0.180	0.213	0.046	0.919	0.297
M-NIKBUT	0.014	-0.147		<.001	0.041	0.068	<.001	0.054	0.483
F-NIKBUT	-0.007	0.022	0.818		0.033	0.195	<.001	0.561	0.976
Bulbar redness	0.035	-0.117	0.178	0.185		<.001	0.013	0.209	0.006
Limbal redness	0.016	-0.109	0.159	0.113	0.811		0.147	0.041	0.013
FBUT	-0.071	-0.174	0.360	0.464	0.215	0.127		0.522	0.101
Corneal staining	0.086	-0.009	-0.168	-0.051	-0.110	-0.178	-0.056		0.028
Conjunctival staining	0.189	-0.091	-0.062	-0.003	0.240	0.217	0.143	0.191	
CCT	-0.197	0.002	0.021	0.004	0.042	0.127	0.106	-0.123	-0.050
TCR_{TMH}	-0.046	0.050	0.017	-0.012	-0.087	-0.084	0.003	0.101	-0.089
TCR_{TMD}	-0.030	0.074	0.036	0.035	-0.105	-0.100	0.018	0.072	-0.091
TCR_{TMA}	-0.058	-0.064	0.049	0.025	-0.102	-0.095	0.045	0.111	0.020
Upper MG drop-out	0.102	0.081	0.049	0.062	0.160	-0.006	0.007	0.070	-0.002
Lower MG drop-out	0.135	-0.161	0.041	0.049	0.184	0.094	0.053	-0.111	-0.029
Upper LWE score	0.067	-0.023	0.002	0.015	0.002	-0.013	-0.017	0.023	0.150
Lower LWE score	0.179	0.019	-0.028	0.011	-0.056	-0.148	-0.105	0.010	0.163

OSDI - Ocular Surface Disease Index; TMH_{K5M} - K5M-based tear Meniscus height; S-TMH - OCT-based static tear meniscus height; S-TMD - OCT-based static tear meniscus depth; S-TMA- OCT based static tear meniscus area; D-TMH - OCT-based dynamic tear meniscus height; D-TMD - OCT-based dynamic tear meniscus depth; D-TMA - OCT based dynamic tear meniscus area; MG - Meibomian glands; FBUT - fluorescein tear film break -up time; CCT - central corneal thickness; LWE - lid wiper epitheliopathy score; TCR - tear clearance rate (TMH-based, TMD-based or TMA-based)

Table 38. Linear correlation coefficients matrix between all the considered ocular measures reported in the longitudinal study and their corresponding P-values

P-value / R coefficient	CCT	TCR_{TMH}	TCR_{TMD}	TCR_{TMA}	Upper MG drop-out	Lower MG drop-out	Upper LWE score	Lower LWE score
Temperature	0.726	0.564	0.552	0.393	0.317	0.668	0.246	0.003
Relative humidity	0.765	0.696	0.698	0.872	0.687	0.244	0.004	0.129
OSDI	0.575	0.905	0.815	0.586	0.070	0.036	0.753	0.076
DEQ-5	0.414	0.575	0.863	0.438	0.025	0.959	0.524	0.585
TMHK5M	0.018	0.128	0.173	0.343	0.787	0.255	0.386	0.352
S-TMH	0.166	0.048	0.047	0.014	0.671	0.059	0.892	0.446
S-TMD	0.636	0.056	0.029	0.014	0.498	0.003	0.973	0.723
S-TMA	0.232	0.274	0.249	0.154	0.419	0.014	0.736	0.620
D-TMH	0.500	0.731	0.812	0.736	0.744	0.529	0.807	0.055
D-TMD	0.523	0.680	0.386	0.182	0.487	0.074	0.244	0.376
D-TMA	0.023	0.601	0.731	0.508	0.245	0.123	0.443	0.039
Tear osmolarity	0.986	0.568	0.399	0.462	0.355	0.065	0.797	0.828
M-NIKBUT	0.815	0.846	0.685	0.574	0.576	0.643	0.984	0.749
F-NIKBUT	0.959	0.894	0.694	0.776	0.481	0.576	0.860	0.898
Bulbar redness	0.633	0.322	0.233	0.247	0.067	0.034	0.979	0.527
Limbal redness	0.145	0.339	0.255	0.280	0.942	0.283	0.887	0.091
FBUT	0.227	0.974	0.837	0.607	0.941	0.548	0.848	0.229
Corneal staining	0.159	0.248	0.409	0.205	0.424	0.206	0.797	0.909
Conjunctival staining	0.572	0.308	0.298	0.821	0.986	0.744	0.087	0.062
CCT		0.573	0.472	0.435	0.139	0.788	0.409	0.835
TCR_{TMH}	0.050		<.001	<.001	0.433	0.937	0.331	0.275
TCR_{TMD}	0.063	0.843		<.001	0.141	0.849	0.605	0.333
TCR_{TMA}	0.069	0.843	0.872		0.801	0.753	0.290	0.994
Upper MG drop-out	0.129	0.069	0.129	0.022		0.029	0.930	0.562
Lower MG drop-out	0.024	-0.007	0.017	0.028	0.190		0.115	0.531
Upper LWE score	0.072	0.085	0.045	0.093	-0.008	-0.138		<.001
Lower LWE score	-0.018	-0.096	-0.085	-0.001	-0.051	0.055	0.311	

OSDI - Ocular Surface Disease Index; TMH_{K5M} - K5M-based tear Meniscus height; S-TMH - OCT-based static tear meniscus height; S-TMD - OCT-based static tear meniscus depth; S-TMA- OCT based static tear meniscus area; D-TMH - OCT-based dynamic tear meniscus height; D-TMD - OCT-based dynamic tear meniscus depth; D-TMA - OCT based dynamic tear meniscus area; MG - Meibomian glands; FBUT - fluorescein tear film break -up time; CCT - central corneal thickness; LWE - lid wiper epitheliopathy score; TCR - tear clearance rate (TMH-based, TMD-based or TMA-based)

CHAPTER IV. CONCLUSIONS AND DISCUSSION

Chapter IV. Conclusions and discussion

The aim of this dissertation is to develop a new macro-type biomarker for supporting early DED diagnosis. The main hypothesis driving this research is that the *loss of homeostasis of the tear film*, describing the core pathophysiological mechanism of DED, may be expressed by the lack of equilibrium between hydrodynamic processes occurring in the tear fluid and tear menisci. Considering the multifactorial nature of DED it was hypothesized that the ocular measure summarizing all the hydrodynamic phenomena occurring in the tear fluid, could potentially become a new macro-type biomarker for supporting DED diagnosis. TTR and TCR are proportional to the sum of all the hydrodynamic phenomena occurring in the tear fluid. Like DED, these markers are multifactorial in nature and have shown their potential in supporting DED differential diagnosis. Several attempts have been made to standardize the procedure and image analysis for TTR assessment after the development of commercially available fluorophotometer. Nevertheless, the prolonged, sophisticated methodology and the requirement for a specialized tool and skills to perform TTR estimation have made the method confined mostly to research setting, with only few clinically applicable alternatives. Therefore, TTR was overlooked as a clinical measure for supporting DED diagnosis. This study introduces new approaches to TTR analysis with emphasis put on their potential clinical application.

4.1. Tear fluorescein wash-out rate estimation - Experiment I

The proposed profilometry-based method of TFWR estimation could be used in a clinical setting as a simple, rapid and easy-to-perform alternative to fluorophotometry. Proposed methodology and algorithm for ESP image analysis can be used to analyse changes occurring in the tear fluid on the whole exposed corneo-scleral surface of the eye. The area of analysis is therefore not restricted to a relatively small area, as it is characteristic of fluorophotometric measurements of TTR. Additionally, due to high spatial resolution of the device, ESP-based estimations of TTR are not biased by the corneal permeability to fluorescein. As the vast part of the image observed with the ESP does not reflect light, the hypothetical situation, where the fluorescent dye is fully absorbed by the cornea, would result in no image being observed. This method is also relatively short and lasts up to approximately one minute, which is much less, even in comparison with the simplified procedure of TTR assessment with fluorophotometry.

Despite being statistically significant, correlation coefficients between TFWR and reported ocular measures are quite low. It is however not an uncommon feature of the ocular measures used for supporting DED diagnosis[8], that are commonly characterized by very low or no correlation between each other. The reported correlations of TFWR with FBUT and DED symptoms assessed with the comprehensive McMonnies questionnaire show its potential as a clinically applicable tool for ocular surface disease diagnosis. The fact that TFWR was correlated with FBUT does not necessarily mean that these two methods are equivalent and could be substituted. Tear dynamics consists of several factors and while FBUT mostly concerns tear film retention on the surface and tear film stability, TFWR relates to tear film distribution, turnover and tear elimination through the nasolacrimal

system. TFWR was not correlated with any other considered measure, including age, tear meniscus height and blink frequency, however because of the tear film being separated from the tear menisci after the blink, it is not a conflicting result. Low correlations may also suggest that TFWR is an independent tear film measure and cannot be directly compared with any of the measures assessed in the ***Experiment 1.*** The use of moistened florets to observe TFWR with fluorescein profilometry ensures sterility and safety. In general practice it is desirable to minimize the instilled volume of fluorescein when assessing tear film parameters. However, even a well-standardized method of fluorescein application with the strip, as performed in this experiment, does not ensure constant concentration and volume of the instilled solution and thus the method of TFWR estimation cannot be considered quantitative. As the introduction of the precise volume of fluorescein could increase repeatability, decrease variability and make the measurements quantitative, it is of interest for the future studies to investigate the performance of the ESP-based method using the standardized volume and concentration of fluorescein used in fluorophotometry-based studies of TTR (1 µL of 2% fluorescein sodium solution). Study aiming to estimate the volume and concentration of the instilled fluorescein solution, while using moistened strips of different surface areas, has shown, that the method of application utilized in the ***Experiment 1*** corresponds to approximately 3 µL of 2% fluorescein topical solution[256]. Additionally, one needs to have in mind that topically applied fluorescein, because of its increased risk of contamination with *Pseudomonas aeruginosa,* has limited availability to optometrists in several European countries, including Poland and Spain. This limitation was mainly the reason why the ESP-based estimation of TFWR was considered qualitative and was not adapted in the protocol of ***the longitudinal study of biomarkers' trends***.

A pilot study performed independently by Biomedical Signal Processing Group at Wroclaw University of Science and Technology has shown that the combination of dye and solute used in this experiment provides optimal coverage without significantly thickening the tear film and allows the observation of stable and undisturbed projection of the ESP diffused pattern. The hyaluronate-based artificial tears are known to be more viscous than the saline-based solutions used to assess TTR, FCT or FBUT, thus they are expected to have different retention times on the ocular surface. During the ESP measurements it is very important for the subject to blink few times to evenly distribute the dye on the ocular surface. If not done so, the nonconfluence of the fluorescent pattern may occur and distort the results. The optimal combination of the fluorescein strips and the eye physiological solution, that results in the best quality of recorded images, is still a matter of investigation and was chosen and tested only for corneo-scleral topography measurements. The combination of fluorescein sodium 1 mg strips with 0.1% sodium hyaluronate should only be taken as some guidance for acquiring good measurements with the ESP, but by no means as a standard procedure.

The repeatability of this method was estimated to be around 14%. TFWR was reported as highly subject-dependent, however it is not a conflicting observation, as this measure potentially considers many factors of tear dynamics like distribution, retention, evaporation, turnover and perhaps also the tear film stability, considering its previously mentioned correlation with FBUT.

Only one out of the two illuminating diodes was illuminated during the measurements to decrease reflex lacrimation in response to excessive radiation. The device was developed for quick measurements with a single flash, thus the intensity of the light coming out from

the two illuminating diodes could be too intense to maintain subject's ocular comfort, even during a relatively short exposure. Reflex tearing could cause the fluorescent dye to spread non-uniformly on the ocular surface, which could impact TFWR estimation.

TFWR values seem to be similar in temporal characteristics to TCRs observed with OCT-based method. Both are presumably a manifestations of an early-phase tear dynamics, as defined in ***Chapter I.*** However, values calculated with these two methods were not correlating with each other. Nevertheless, is not a conflicting result, which comes from the fact that shortly after each blink, the pre-corneal tear film becomes physically isolated from the tear menisci and the diffusion between these two tear compartments does not occur[186,187]. Thus, even though these two methods share temporal exponential characteristics, they may be following different hydrodynamic phenomena. Therefore, the group mean of TCR and TFWR obtained with these experiments are not comparable. Artificial tears-based solution used in the ESP-based method is more viscous than the saline solution used in TCR-based measurements with OCT and thus it has different temporal wash-out characteristics. Both curves of tears exchange: the ESP-based fluorescein intensity decay curve and the OCT-based tear meniscus height decay curve, are exponential. Perhaps an indication for the future studies of tear exchange would be to investigate changes in TFWR and TMH dynamics simultaneously, to observe TFWR and TCR as a single measure of tear exchange. Firstly, a study on large cohort should be performed to define mean values and thresholds between symptomatic and asymptomatic subjects and specify the strict amount and concentration of the fluorescent dye necessary to perform quantitative assessment of TFWR.

Summarizing, the profilometry-based measurement of TFWR provides a mode for assessing subtle changes in tear film dynamics. Future studies should investigate applicability of this technique on more severe cases of DED and standardizing the method of fluorescein instillation for quantitative assessment.

4.2. Limitations of the longitudinal study

This study was the first independent study of the long-term effects of modern daily disposable contact lens wear. The idea behind choosing daily disposable contact lenses for this experiment was to induce and observe changes in ocular physiology, without putting subjects at risks associated with long-term contact lens wear. Simplified lens care and hygiene alleviate the risk of infection whereas daily disposable modality facilitates subjects' adherence to wearing schedules.

However, some may say that the limitation of the study is that the lens type was not masked from the observer fitting the lens, which could result in some potential bias in choosing the appropriate contact lens for the subjects. The author of this thesis agrees that randomization is a very important aspect of clinical trial design. However, contact lens fit assessment was performed by two skilled professionals and following a strict protocol for contact lens fit, to provide subjects with the best lens possible from the available options. Choice of the lens was based on quantitative measures of contact lens fit and objective measures, like NIKBUT. Applied interventions could not be randomized, in a sense that half of the group could be fitted with one lens, and the other half with the other lens. Contact lens assessment was double-checked by two practitioners, who agreed on the lenses, additionally considering subjects' comfort. The practitioners did not have any interest in choosing any of the lenses over the other, that could go beyond this protocol and the subject were masked

from the lens type while they were comparing the comfort of the contact lenses. In the case of this study, randomly chosen lenses increase the odds of wrong fit, which may result in potential risks to participants.

It could be of interest to increase the number of Hy wearers to match the number of SiHy wearers, however it was not possible, due to the time limitation of the EDEN project and non-randomized nature of contact lens fit. Nevertheless, comparing two types of contact lenses was not the aim of this investigation and, since there was no statistically significant difference noted in any of the ocular measures assessed in the study between SiHy and Hy-fitted group, it was concluded that lenses have achieved similar performance and have affected ocular physiology in a similar manner. Additionally, for all the ocular measures, which were assessed binocularly, there was no statistically significant difference observed between right and left eye during the initial Baseline visit. Therefore, all subjects were unified and analysed as one cohort of refitted contact lens wearers.

Second limitation was related to statistically significant differences in environmental conditions (temperature and relative humidity in the laboratory) reported between different sessions of the longitudinal study. These differences may be due to changes of the seasons and could not be avoided. The environmental triggers could possibly influence tear osmolarity measurements, as the impedance-based osmometer measurements are temperature-dependent. However, for each subject, the minimum and maximum difference in temperatures across the time-course of the study were only 1.9°C and 5.0°C, respectively. Corresponding differences in relative humidity were 9.7 %RH and 27.6 %RH, respectively. Additionally, changes in tear osmolarity do not seem to correspond to changes in temperature and humidity, since osmolarity trends are characterized by a steady decay,

while the temperature and relative humidity fluctuate around the year. Subjects had an environmental adjusting period if they arrived in the laboratory from the outdoors. Almost none of the ocular measures considered in the study was shown to correlate linearly with environmental characteristics, except statistically significant, low negative correlation between the lower LWE score and temperature and positive correlation between the upper LWE score and relative humidity (see Tables 36-38). No subjects have been excluded from participation in the study because of reported adverse effects of contact lens wear. Few subjects have resigned for health-unrelated reasons (lack of time) and one because of pregnancy.

4.3. Reported symptoms

The compliance of subject is an important factor that should be considered when analysing the reported symptoms. Free supply of lenses aided attendance outcomes and following wearing schedules, which made the study design more robust, however providing free contact lenses could bias the results of OSDI and DEQ-5 questionnaires as inadvertently the Hawthorne effect might have been introduced[257]. Hawthorne effect describes the situation in which subjects modify an aspect of their behaviour, in this case, their answers to the questionnaires, in response to their awareness of being observed, in this case, being regularly checked by an eye care professional. This may be the reason for the statistically significant decrease in OSDI after Baseline visit. Subjects were responding well above average for newly-fitted contact lenses. Further investigation of subjects-reported symptoms has shown that two distinctive groups of subjects can be observed - one, which was initially reported as symptomatic and the other, that was asymptomatic at the Baseline visit. This division was based on a standardized threshold between non -and mild DED

reported with OSDI and DEQ-5. For subjects initially reported as symptomatic, a substantial decrease in symptoms was reported after Baseline. Perhaps, these subjects were more prone to Hawthorne effect, being extra motivated to wear contact lenses that were well-fitted and more comfortable than their habitual lenses. For the initially asymptomatic group OSDI was slowly increasing and reached statistical significance at 6-month visit. Thus, changes in symptoms may suggest, that while contact lens wear induces slight worsening of symptoms in asymptomatic subjects, at the same time, it has the potential to alleviate symptoms in symptomatic contact lens wearers, considering that lenses are properly fitted, and the subjects are following a systematic schedule.

Major drop of OSDI was reported at the Control visit, which may suggest that symptoms reported with OSDI later in the study were caused by contact lens wear. Statistically significant change in symptoms was not reported with the DEQ-5 questionnaire, which additionally suggests, that these symptoms where not DED-specific. DEQ-5 score of more than 5 may be an indicator of DED, thus from results reported in the study, it seems that the subjects started the study with generally mild DED symptoms and most of them had finished the study without any DED symptoms.

OSDI returned to the baseline value after six months. This drop in OSDI could be attributed to difference between current and previous lens wear modalities as well as the difference between lens material or perhaps, in subjects' adherence to wearing schedule. Most likely, it was a positive effect of lens refitting that has been observed. Some studies report the positive effect of contact lens refitting across different materials and modalities[258-260]. This assumption will be discussed later in this Chapter, while reporting tear osmolarity measures.

Additionally, this research provides a typical example of a very common problem in DED diagnosis, which is the general lack of correlation between ocular sings and reported symptoms. Statistically significant linear correlations with reported symptoms were observed between OSDI and lower eyelid Meibo-score, OSDI and FBUT and between DEQ-5 and upper Meibo-score (see Tables 36-38). Most of these values were relatively constant over the time-course of the study. These correlations, however, are very low, even though statistically significant. Naturally, the scores obtained from two questionnaires were positively, linearly correlated with each other. Other objective measures of ocular physiology described below should be unaffected by the Hawthorne effect.

4.4. Non-invasive Keratograph tear film break-up time

Tear film, which is divided by the contact lens into two layers, undergoes biophysical and biochemical changes, thus contact lenses can influence tear function and impact wearer's comfort. The use of an automated, objective protocol to acquire NIKBUT alleviates the risk of bias of tear film break-up time estimation. The use of infrared radiation minimizes the risk of reflex tearing. The reported measures of NIKBUT have shown, that the estimates of the pre-lens tear film surface quality acquired at Day 2 visit were not significantly different to those recorded at following visits. This may suggest that modern daily disposable lenses have reached such a level of performance, that allows them to minimally impact tear film surface quality and tear film break-up. Thus, as it was pointed out by Mousavi et al.[39], measurement of the pre-lens tear film surface quality reflect tear film stability reported after longer periods of wear[261,262].

Statistically significant changes in NIKBUT were noted between Baseline and other visits. The differences in NIKBUT recorded between Baseline and Control visit were most likely

spurious, since they were reported only for one eye and with low statistical significance (P = 0.048). Difference between pre-corneal and pre-lens NIKBUT was observed at the beginning of the study, however modern daily disposable lenses, when properly fitted and worn with adherence to more moderate schedule, do not seem to further disturb the pre-lens tear film surface quality. There was no clinically important difference in tear film surface quality attributed to the lens surface treatment or material, as there was no statistically significant difference reported between two types of fitted lenses in any of the assessed ocular measures.

4.5. Tear osmolarity and healthier contact lens wearing habits

The hypothesis was that wear of modern daily disposable soft contact lenses will maintain or increase tear osmolarity over the time-course of the longitudinal study. DED in contact lens wearers may be explained by increased tear film thinning resulting in increased tear osmolarity[263] or by the loss of corneal sensation particularly associated with long-standing wear of hard and extended-wear contact lenses[50,264]. Lowered corneal sensitivity leads to decreased tear secretion in response to reflex stimuli, presumably causing an increase in tear osmolarity[264]. Extended-wear soft contact lenses can decrease corneal sensitivity more than daily-wear soft contact lenses[264]. Generally, it has been suggested that contact lens wear increases tear osmolarity, particularly in changing environmental conditions[263,265]. Contrarily to those works, this study reported statistically significant decrease in tear osmolarity with contact lens wear. As could be observed based on subject-reported symptoms, modern daily disposable contact lenses may not necessarily lead to typically known adverse effects, such as ocular discomfort or inflammation of the ocular surface[64,266].

The observed decrease in tear osmolarity is probably not associated with seasonal changes in temperature. As noted earlier, those environmental changes, although significantly different among visits, were not substantial for each of the subjects. Khanal and Millar found no correlation between tear osmolarity measurements and temperature or humidity[67]. Hence, these two environmental factors do not have such high influence on tear osmolarity measurements and most probably could not cause such substantial changes in this parameter, as the ones reported in the ***Results*** section of ***Chapter III.*** The temporal differences in tear osmolarity are likely a function of the subjects' ability to maintain tear film homeostasis, overcoming the drying effect of contact lenses[46]. Nevertheless, tear osmolarity was never shown to decrease in contact lens wearers in any of the studies reported in relevant scientific literature. Tear hyperosmolarity has been reported in daily and extended wear of both soft and hard contact lens wearers[267-269], especially in symptomatic subjects[263,268].

All participants were refitted based on three main criteria – contact lens fit, subjective comfort and tear film surface quality, as reported in the study of Mousavi et al.[39]. This way of refitting, additionally including a control visit after 2-weeks, ensures good lens performance. Newly-fitted lenses decreased tear osmolarity in all participant, regardless of the contact lens material. One major aspect that should be taken into consideration is that in Poland where this research was conducted there are practically no restrictions put on contact lens choice. Lenses are available without prescription and easily accessible from multiple sources, offering competitive prices. Many other countries have similar contact lens market. This leads to increased number of self-fitting with more affordable options, with subjects usually choosing monthly, extended-wear economic solutions over daily disposable lenses. This is supported by the fact that 54% of the study participants had worn

monthly and 25% of them had worn fortnightly soft contact lenses before commencing the study. The abovementioned behaviours may lead to poor fitting decisions, lack of follow-up by an eye care professional and perpetuating risky habits. Additionally, most of the subjects taking part in the study were initially reported with visible shortage of Meibomian glands, which may suggest long-term contact lens wear[270]. Additionally, the mean value of OSDI reported at Baseline visit of the longitudinal study (13.9 ± 11.9 [-]) was higher than the threshold reported for healthy individuals (OSDI < 13 [-]), which also can be due to prolonged contact lens wear in the past.

Thus, the effect of contact lens wear on tear osmolarity may not be attributed entirely to the lens modality, but also to generally healthier (than habitual) contact lens wearing habits, more moderate wearing schedule and appropriate contact lens fit and control. Moreover, subjects were becoming more responsible for their ocular health knowing that they will be regularly checked by an eye care professional and that the lack of adherence could get them excluded. This may have caused a subtle bias in reported symptoms (the abovementioned Hawthorne effect), however could not possibly impact objectively-measured ocular sings, including tear osmolarity. Subjects with high initially reported symptoms were not excluded from participation, since no accompanying signs of severe dry eye was reported, and no adverse effect of contact lens wear was observed over the time-course of the study.

Subjects could wear their newly-fitted lenses only five days a week and not more than 12 hours per day, thus the effect of the lens itself may be modified by changing to a more moderate wearing schedule. Two days of break in contact lens wear and simplified contact lens hygiene may have added to the total decrease in signs and symptoms, including tear osmolarity. Moreover, no statistically significant difference between SiHy and Hy-fitted

group in any of the ocular measures assessed in the study may suggest that the lens material may be adding to this decrease less than the lens modality and subjects' adherence to guidelines. This study shows that the longitudinal effect of modern daily disposable soft contact lens wear, and moderate wearing schedule may prove beneficial to some subjects, especially those who were initially reported with hyperosmotic tears or ocular surface disease symptoms. Statistically significant difference in tear osmolarity between Baseline and Control visit shows that low osmolarity was maintained after the study. Even subjects who exhibited highly osmotic tears returned to tear osmolarity values reported for non-wearers[269]. Studies show that some ocular signs of DED are less prevalent among wearers of daily disposable lenses[271] and subjects may benefit from changing their conventional reusable daily wear lenses to daily disposables[272]. A positive effect of refitting with modern daily disposable contact lenses on tear osmolarity was independently assessed in this dissertation. Moreover, tear osmolarity proved to be sensitive enough to track subtle changes in ocular physiology in healthy, young subjects, thus it is expected to show more pronounced differences in DED subjects. Tear osmolarity may prove to be a good macro-type biomarker in supporting ocular surface disease diagnosis and a marker of response to an effective therapy.

4.6. Meniscometry and meniscus dynamics

Spectral-domain OCT-based meniscometry has shown good intra-observer and inter-observer repeatability and allows following changes of tear meniscus morphology during blinking and after topical instillation. However, Bartuzel et al. indicated that any observed changes in the early post-blink phase meniscus parameters must be viewed with caution, as they are most likely related to the longitudinal movement of the eye rather than to the post-blink tear meniscus formation, corresponding to tear film build-up, as commonly stated in the past. Additionally, the geometrical parameters of the tear menisci may depend on many biophysical and external factors and while the environmental impact on meniscometry can be controlled and maintained, the subject-related aspects are difficult to control. OCT-based meniscometry provides non-invasive and more in-depth visualization of tear menisci and the image acquisition is rapid and simple. However, the analysis of the acquired images may be complex, time-consuming and operator-dependent. Thus, the computer program allowing dynamic image analysis to minimize interfering factors related to head, eye and eyelid movements was developed[200] and used in the ***Experiment II*** and ***III*** to assess the OCT-based meniscometry and temporal changes of tear meniscus parameters after topical instillation for TCR estimation.

The experimental study of dynamic meniscometry aims to follow morphological changes in the OCT-based image of the eyelid junction. The Bland-Altman and scatter graph shows that the automatic method of meniscometry based on 90 OCT B-scans gives relatively higher TMH estimations than the meniscometry assessed based on a static OCT image, especially for higher values of TMH. However, TCR estimates assessed with OCT are generally higher than the TTR values assessed with fluorophotometry. It is probably

because of fluorophotometry being a highly sensitive device, that can detect fractions of fluorescein in the tear film. Additionally, the OCT-based TCR is most likely related to an early-phase, rather than to the basal, slow phase of tear turnover.

One can observe, that while the D-TMH decreases significantly in the beginning of the study, the other geometrical parameters of tear meniscus do not. D-TMD and D-TMA were observed to stay constant or increase insignificantly. This may imply several different conclusions. One is that the tear meniscus or the conjunctival sac has changed its shape over the time-course of the study, or the depth of the eyelid junction had increased. As it was shown by Wolffsohn et al., contact lens wear may cause indentation of the ocular surface, which depends on midperipheral contact lens shape and edge characteristics[273]. Therefore, it is possible that contact lens wear had induced changes in tear meniscus and conjunctival sac morphology. A careful observer can notice, that the increase of tear meniscus height after saline solution instillation during the OCT-based assessment of TCR is decreasing over the time-course of the study. To better visualize this 'change in increase', tear meniscus height dynamics, presented earlier were normalized and provided in the Figure 39. As one can see, even if the amount of fluid instilled into the conjunctival sac was kept constant over time, the potential amount of fluid that can enter the subjects' tear meniscus (which is expressed as the increase in D-TMH at '0' minutes post-instillation) is decreasing over the time-course of the study. (See figure 39)

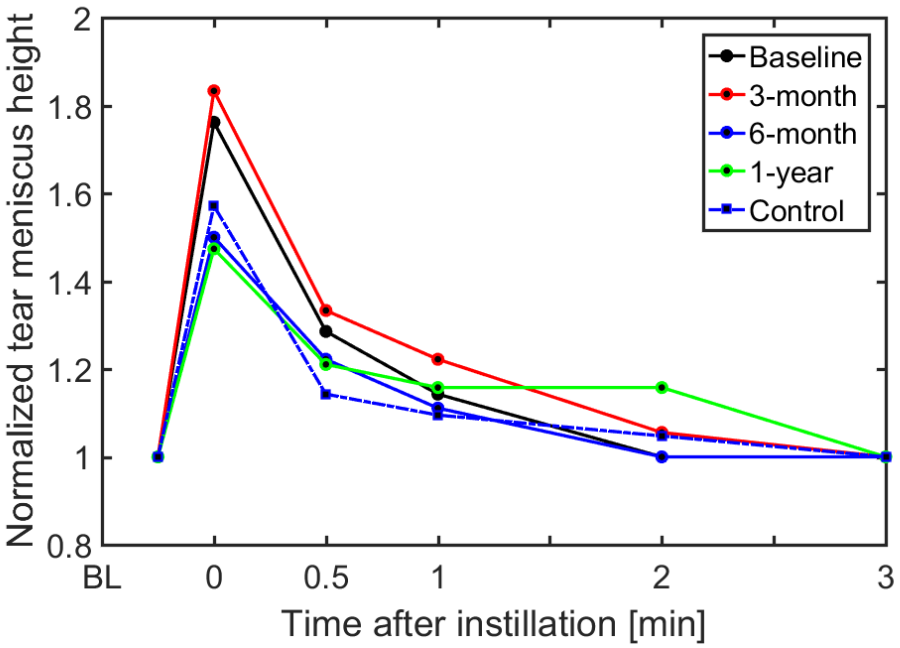


Figure 39. Normalized tear meniscus height dynamics

The same characteristics were observed for two other geometrical measures of tear meniscus (D-TMD and D-TMA). This decrease occurs when contact lenses are fitted, to slightly moving toward its basal form at Control visit, suggesting a short-term effect induced by contact lens wear. The question is whether this decrease was caused by changes in tear dynamics and delay of the Krehbiel flow or by changes in the conjunctival sac or anterior surface morphology.

Changes in the corneo-scleral topography were beyond the scope of this research and changes in the tarsal conjunctival thickness cannot be observed with available devices. The corneal thickness however could provide an etiological clue to whether any changes in the anterior eye surface morphology occurred. Statistically significant changes in CCT were reported, particularly between 12-month and any other visit, except at 6-month visit. CCT was shown to slightly decrease. These changes in CCT correspond with the jump in

tear meniscus parameters. Moreover, D-TMA was reported to negatively linearly correlate with CCT, conjunctival staining and lower LWE scores (see Tables 36-38). This may all suggest that morphological changes of the anterior surface and tarsal eyelid could have caused the tear meniscus to change its shape.

There is no direct proof that these changes had occurred due to a slight shift in tear meniscus dynamics, or changes in anterior surface morphology or both, however considering changes in tear film physiology measures observed throughout the whole duration of the study one can conclude that the shape of the anterior ocular surface may have occurred and may have influenced the central TMH. If the tear volume was constant over the time-course of the study and has been redistributed from the central to the temporal and nasal compartments of the tear menisci, then it could cause a steady decrease in tear osmolarity in the absence of increasing central tear meniscus and the delay of tear clearance. Samples for tear osmolarity measurements were collected from the temporal part of the tear menisci, where the tear fluid was accumulating due to ocular surface indentation. This observation also opens the possibility for future exploration of corneal shape parameters and their effect on the tear meniscus morphology. Whatever the reason, this seems to affect the TCR.

This study shows the importance of visualizing tear meniscus with in-depth OCT-method and using a sensitive, automatic, objective protocol. The practitioner was not masked on subjects, thus the use of objective, automatic protocol guarantees unbiased image analysis. Development of an automated image processing software to detect and measure tear meniscus, removes the potential bias and errors in judgment, making this dynamic method of tear meniscus assessment objective, simple and clinically applicable.

The impact of soft contact lens wear on the cornea and its physiology is well-studied, however little is known about the potential changes in the tarsal conjunctiva due to contact lens wear. Contact lens alternates the tear film layer, decreasing its thickness and stability. It limits the ability to maintain adequate lubrication and minimize friction between two ocular surfaces during blinking. Increased number of giant papillary conjunctivitis[274] was noted among contact lens wearers. Additionally, contact lens wear was associated with lid parallel conjunctival folds[275] and Meibomian glands dropout[270,276]. Few studies have investigated lid wiper staining during lens wear or examined specifically the links between signs and symptoms of contact lens discomfort.

Additional reason why the D-TMH was shown to decrease, without substantial change observed in D-TMD and D-TMA, is that the contact lens had somehow influenced the composition of the tear meniscus, causing it to change its refractive properties. While this change in the refractive index does not influence the tear meniscus height, it can influence the way in which the air-tear meniscus interface refracts light coming from the OCT, and thus it could influence the tear meniscus depth and area in a way that cannot be compensated by the OCT's built-in software. Hence, future studies should investigate the potential impact of contact lens wear on tear composition and the refractive index of the tear film and menisci.

Summarizing, a downward trend of tear meniscus height was observed. This could be caused by corneal or conjunctival deswelling, by changes in tear meniscus shape due to ocular surface indentation by the contact lens, by changes in the lid wiper, or changes in the refractive index of the tear meniscus fluid. Corneal swelling was not reported in this study, contradictory, the central corneal thicknesses was reported to decrease over time.

4.7. Tear Clearance Rate

This study shows that the OCT-based TCR is decreasing during contact lens wear, probably due to lower baseline tear meniscus values and decreased lower conjunctival sac volume in its central part. Here, unlike in any other studies of TCR, the tear meniscus parameters were assessed based on dynamic meniscometry. D-TMH, D-TMD and D-TMA measurements were taken into consideration when calculating TCRs. All observed images were clear enough to be analysed with the provided software. As explained in ***Introduction,*** in most of the kinetic studies of TTR with fluorophotometry, the biphasic characteristics of fluorescein intensity decay can be observed, with faster phase occurring just after fluorescein instillation and slower, basal phase, occurring after 5 minutes post-instillation. Findings of Zheng *et al.*, as well as the findings presented in the experiment suggest that TMH decreases most significantly at the early phase post-instillation. Zheng et al. suggested the effect of Krehbiel flow on TCR estimations and showed that TCR assessed with OCT is likely the manifestation of an early-phase tear dynamics. Contributing to these developments, presented research has shown that the estimated time for the tear meniscus to come back to its basal level after the instillation of 5 μL of saline solution was about 2-3 minutes in young healthy subjects. The mean TCR was estimated as (mean ± standard deviation) 29 ± 13 %/30s compared to 35 ± 11 %/30s for younger group reported by Zheng et al. and showed large variation between subjects, with relatively good reproducibility. In both studies of Zheng et al. the age-related differences in TCR were evident, noting a wide age gap between tested groups of subjects (with TCR estimated as 35.2 ± 11%/30s versus 12.4 ± 7.3%/30s for younger [29.6 ± 7.2 y/o] and older group [71.4 ± 10.8 y/o], respectively). This study attempted to test the improved OCT methodology in a group of subjects with narrower age range.

An additional functionality that should be considered in the future studies of tear clearance with OCT is to control the amount of blinks between each measurement or to assess the TMH in a continuous manner, as it was observed by Wu et al.[277]. In various studies it was shown, that the blink rate influences TTR[122]. This study shows statistically significant linear correlation between TCR and blink frequency. It could be also of interest to differentiate blinks of different temporal characteristics and quality, e.g. full blinks form partial ones. The author of this dissertation believes that controlled blinking frequency could disturb the natural process of tear exchange. However, taking into consideration the expected impact of blink frequency on the results, it could be of interest to measure it simultaneously with TCR. Future studies should investigate both the applicability of these techniques on DED subject and the qualitative differences in early-phase tear dynamics in subjects with symptomatic DED[104].

Summarizing, using OCT can be rapid, qualitative and quantitative method of determining TCR. With the new software developed, tear meniscus parameters can be calculated more precisely, considering the nonconfluence of tear meniscus morphology following each blink and small eye movements. Traditional clearance tests are either invasive, laborious or indirect and fail to follow dynamic changes occurring in the tear film and menisci. OCT-based method of TCR estimation is non-invasive, relatively shorter and simpler to perform, than the traditionally used tear clearance tests. Additionally, this or similar technique could be used in testing artificial tears performance[214,215]. TCR is proven to decrease in both elderly and symptomatic subjects. TCR also correlates with age. Apparent, statistically significant, yet small correlation reported in the ***Experiment 3*** with age is the result of a narrow age group. It comprised mostly of young, healthy individuals. Newly developed software allows precise, automatic estimation of tear meniscus parameters.

It is worth noting, that most of the subjects taking part in the experiments and in the longitudinal study were habitual contact lens wearers, thus their eyes were exposed to contact lens-related changes on the ocular surface. This might have decreased their TCRs and tear meniscus parameters, which makes the reported Baseline value lower than the one reported by Zheng. Also, the use of an automatic analysis protocol instead of measuring tear meniscus based on singular static images, might have contributed to this difference. TCRs reported in the experiment are still higher than the ones reported by Zheng for older subjects. TCR estimates assessed with OCT are generally higher than the TTR values assessed with fluorophotometry. At 30 second margin after instillation, D-TMH decreases in a larger rate compared with D-TMD (21 ± 20%/30s compared with 18 ± 18%/30s).

As mentioned, the animation sequence contains 90 B-scans and the time when the subjects must keep their eyes open is usually less than 3.75 seconds. In all subjects this amount of time was not associated with any observable or reported effort. Severely affected DED subjects could have a problem to keep their eyes open for this period. However, one should take note that the number of B-scans can be reduced. A pilot study testing the new algorithm of dynamic meniscometry revealed that as little as 20 B-scans was shown to be enough for providing a reliable estimate of tear meniscus parameters.

In some subjects the negative values of TCR were reported, suggesting that in some cases the tear meniscus parameters were increasing after instillation. This may suggest the delay in Krehbiel flow in these subjects and was the main reason why the TCR values were variable. However, it was not accompanied by reflex tearing, since the tear meniscus was not constant and was decreasing with time in a pace characteristic to tear clearance.

Perhaps, it would be of interest to identify the moment of this delay as a potential marker of tear clearance or consider changing the time margin from 30 seconds to one minute.

As one of the very few measures assessed, TCR was not reported to correlate with any other sing or symptom, except for the dynamic changes in tear meniscus parameters and, naturally, other measures of TCR. These several correlations were expected, as the tear clearance rate is calculated based on dynamic changes of tear meniscus geometrical parameters. Therefore, measurements of the inferior tear meniscus may provide insight into tear meniscus dynamics. TCR may provide an additional measure of tear fluid dynamics, which cannot be expressed by any other ocular measure used in this study.

Singular statistically significant difference was noted between SiHy and Hy-fitted group at Control visit in TMH-based TCR. This may suggest that changes induced by two different lenses were diminishing in different rates after refraining from contact lens wear. The values of TMH-based TCR noted at the Control visit are higher for Hy-fitted group, Therefore, one can assume that Hy-fitted group's TCR_{TMH} was recovering faster than the one reported for SiHy-fitted subjects. However, considering the general lack of statistically significant difference between SiHy and Hy-fitted group in any of the reported ocular measures over the time course of the study, this singular difference may be spurious. Lenses had similar edge characteristics, however Hy lenses are generally thicker than SiHy lenses, which may have added to this difference.

4.8. Changes induced in the ocular surface

Statistically significant changes in Meibomian gland drop-out, LWE score of both eyelids and ocular surface staining may suggest that changes in the anterior eyelid morphology and tarsal eyelid have occurred over the duration of the study. Lack of statistically significant differences in bulbar and limbal redness suggest that these changes were not inflammatory. This may further contribute to the hypothesis that observed changes in tear osmolarity, TMH and TCRs are due to changes in the anterior surface of the eye and the tarsal area, which could be potentially induced by contact lens wear.

Statistically significant changes were observed in conjunctival staining score over the time-course of the study, but only for the right eye. A slight increase in conjunctival staining was noted, however reported values were below the clinical levels reported for healthy subjects. These changes were subtle and most likely caused by contact lens handling while taking the lenses off before each session. Visible fingerprints and localized staining provided a visual clue to whether these changes were induced by the subjects. Additionally, conjunctival staining came back to its basal level at Control visit, which suggests that these changes were short term and most likely related to contact lens wear. Ocular surface staining in this study was mostly used for sanity check and the adverse effect of contact lens wear was not observed in any of the subjects. Increase in ocular surface staining with contact lens wear is a well-known phenomenon, however, when lenses are properly fitted, subjects adhere to wearing schedules and are not allergic to contact lens materials or solutions and the properly handle their lenses, there risk of ocular surface staining is minimized radically. Modern, daily-disposable soft lenses should not induce any adverse surface reactions on the ocular surface. Ocular staining was mainly caused by subjects

touching the globe while taking off or putting on their lenses. The same statistical dependence was noted for corneal staining with fluorescein, however the values of corneal staining were way below the clinical levels reported for healthy individuals.

The Meibomian gland drop-out slightly increased over the time-course of one year. This may suggest that one year of contact lens wear, even accompanied by healthier contact lens wearing habits and adherence to moderate wearing schedule can still induce changes in Meibomian glands structure and statistically significant shortage of the glands. Contact lens wearers are most likely to report with visible shortage of Meibomian glands, accompanied by changes in lipid layer thickness. This should be taken into consideration when fitting contact lenses to subjects with visible shortage of Meibomian glands, since changes induced in Meibomian glands by the contact lens are, up to date, considered irreversible and the functionally of the existing glands can be only partially restored e.g. with thermodynamic treatment.

4.9. Summary

Even though not expected, an evident beneficial long-term effect of changing the contact lens wearing scheme and modality on the tear film and ocular physiology was induced. Biomarkers' trends followed the opposite trend than it was initially projected. This gave the opportunity to observe and identify biomarkers sensitive enough to follow the trends in ocular physiology, that could potentially be indicative of the effective refitting. As these parameters can be objectively, non-invasively and automatically measured, they fulfil the definition of a biomarker, provided in ***Chapter I***.

It has been shown that tear osmolarity may bare potential in supporting DED diagnosis. Tear osmolarity corresponds with changes in ocular physiology because of contact lens re-fitting. It was decreasing for the whole duration of the study and all changes were statistically significant. Even though the reported trends in tear osmolarity were different from initially expected, they were expressing the generally observed positive trends in most of the other ocular measures assessed in the study. Thus, a positive effect of refitting with modern daily disposable contact lenses was independently assessed in this dissertation. Moreover, chosen biomarkers of DED were proven to be sensitive enough to track subtle changes in ocular physiology in healthy, young subjects, thus they are expected to show more pronounced differences in DED sufferers. Tear osmolarity, TCR and TMH may all prove to be good macro-type biomarker in supporting ocular surface disease diagnosis and markers responding to effective therapy.

The OCT-based dynamic meniscometry was shown to respond to very subtle changes in tear meniscus parameters over the time-course of the study, that could not be observed with traditionally used methods of tear meniscus evaluation. These changes could not be

observed with the static technique, based on a single B-scan or with the traditional *en face* measurements. As the desiccating effect expressed by decreased tear volume was not observed in this study, these changes were most probably corresponding to changes in eyelid junction morphology, indentation of the ocular surface area with the contact lens, or by changes in the tear meniscus refractive index. Additionally, the decrease in TCR provided a clue to why the tear meniscus volume seems to decrease in contact lens wearers in the absence of any other negative changes, except for the slight ocular surface staining (connected with contact lens handling) and increase in Meibomian glands drop-out.

Based on the hypotheses of this dissertation, the study aid to understand the role of some ocular biomarkers, particularly tear clearance rate, tear meniscus morphology and tear osmolarity in DED aetiology and had introduced new ways for non-invasive, objective tear film dynamics assessment, that can provide practitioners with clinically valuable information, which can aid DED diagnosis. These biomarkers are sensitive enough to follow subtle changes occurring in the tear film in healthy subjects and hence, in the future, could be utilised for supporting DED diagnosis to follow and predict the progression of the disease.

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APPENDICES

Appendix 1. McMonnies questionnaire

Please answer the following by underlining the response most appropriate to you.
Age: under 25 years 25-45 years over 45 years

Currently wearing: no contact lenses hard contact lenses soft contact lenses

Have you ever had drops prescribed or other treatment for dry eye?
Yes (2) No (0) Uncertain (1)
Do you ever experience any of the following symptoms? (Underline)

Soreness (1) 2. Scratchiness (1) 3. Dryness (1) 4. Grittiness (1) 5. Burning (1)

How often do your eyes have these symptoms? (Underline any that apply to you)
Never (0) Sometimes (1) Often (2) Constantly (3)

Do you regard your eyes as being unusually sensitive to cigarette smoke, smog, air conditioning, central heating?
Yes (2) No (0) Sometimes (1)
Do your eyes easily become very red and irritated when swimming in chlorinated fresh water?
Not applicable Yes (2) No (0) Sometimes (1)

Are your eyes dry and irritated the day after drinking alcohol?
Not applicable Yes (2) No (0) Sometimes (1)

Do you take (please underline): antihistamine tablets (1), antihistamine eye drops (1). Diuretics (fluid tablets) (1), sleeping tablets (1), tranquilizers (1), oral contraceptives (1), medication for duodenal ulcer (1) or digestive problems (1) or for high blood pressure (1) or_____ (1)

Do you suffer from arthritis?
Yes (2) No (0) Uncertain (1)

Do you experience dryness of the nose, mouth, throat, chest or vagina?
Never (0) Sometimes (1) Often (2) Constantly (3)

Do you suffer from thyroid abnormality?
Yes (2) No (0) Uncertain (1)

Are you known to sleep with your eyes partly open?
Yes (2) No (0) Uncertain (1)

Do you have eye irritation as you wake from sleep?
Yes (2) No (0) Uncertain (1)

Appendix 2. Dry eye symptoms questionnaires

Have you experienced any of the following *during the last week*:	**All of the time**	**Most of the time**	**Half of the time**	**Some of the time**	**None of the time**	
1. Eyes that are sensitive to light?	4	3	2	1	0	**N/A**
2. Eye that feel gritty?	4	3	2	1	0	**N/A**
3. Painful or sore eyes?	4	3	2	1	0	**N/A**
4. Blurred vision?	4	3	2	1	0	**N/A**
5. Poor vision?	4	3	2	1	0	**N/A**
Have problems with your eyes limited you in performing any of the following *during the last week*:						
6. Reading?	4	3	2	1	0	**N/A**
7. Driving at night?	4	3	2	1	0	**N/A**
8. Working with a computer or a bank machine (ATM)	4	3	2	1	0	**N/A**
9. Watching TV?	4	3	2	1	0	**N/A**
Have your eyes felt uncomfortable in any of the following situations *during the last week*:						
10. Windy conditions?	4	3	2	1	0	**N/A**
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	**N/A**
12. Areas that are air conditioned?	4	3	2	1	0	**N/A**

1 Questions about EYE DISCOMFORT:						
A. During a typical day in the past month, **how often** did your eyes feel discomfort?	**0 Never**	**1 Rarely**	**2 Sometimes**	**3 Frequently**	**4 Constantly**	
B. When your eyes felt discomfort, **how intense was this feeling of discomfort** at the end of the day, within two hours of going to bed	**Never Have it**	**Not at All Intense**			**Very Intense**	
	0	**1**	**2**	**3**	**4**	**5**

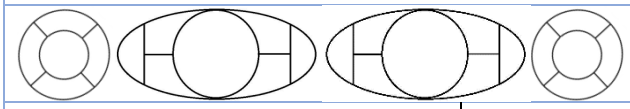
2 Questions about EYE DRYNESS:					
A. During a typical day in the past month, **how often** did your eyes feel dry?	**0** **Never**	**1** **Rarely**	**2** **Sometimes**	**3** **Frequently**	**4** **Constantly**
B. When your eyes felt discomfort, **how intense was this feeling of dryness** at the end of the day, within two hours of going to bed	**Never Have it** **0**	**Not at All Intense** **1**	**2**	**3**	**4**	**Very Intense** **5**
3 Questions about WATERY EYES:					
During a typical day in the past month, **how often** did your eyes feel dry?	**0** **Never**	**1** **Rarely**	**2** **Sometimes**	**3** **Frequently**	**4** **Constantly**
4 Questions about IRRITATED EYES:					
During a typical day in the past month, **how often** did your eyes feel irritated?	**0** **Never**	**1** **Rarely**	**2** **Sometimes**	**3** **Frequently**	**4** **Constantly**

Have you ever had a previous clinical diagnosis of dry eye? Yes ☐ No ☐

Appendix 3. Medical history chart

Date (First visit):		Time:	
Occupation:		Px ID:	
Surname:			
Forename:			
Date of Birth:			
Telephone number:			
Refracting Correction: OD	VA	OS	VA
Distant Vision:			
Near Vision:			
General Health:			
Ocular Health:			
Medication:			
Allergies:			
Last Eye Examination:			
Last Medical Examination:			
Family Ocular History:			
Family Medical History:			
Driver:			
Visual Display Unit (TV, computer…) (how many hours per day):			
Hobbies:			
Smoker:			
Current Contact Lens history: OD: How often:		OS: How long:	
Comments			

Appendix 4. Slit lamp examination protocol

	ID: …… Date:……	
TEAR FILM Qualitative assessment	**OCULUS DEXTER**	**OCULUS SINISTER**
	Good / polluted / excessive lipid / watery / reflex tearing / artefacts / no artefacts / foam / other:	Good / polluted / excessive lipid / watery / reflex tearing / artefacts / no artefacts / foam / other:
TEAR MENISCI	Even / uneven / reflex tearing / low volume/ other:	Even / uneven / reflex tearing / low volume/ other:
BLINKING	Complete / forced / tic / lid inversion / other: …… INCOMPELTE BLINKING:	Complete / forced / tic / lid inversion / other: …… INCOMPELTE BLINKING:
LIDS AND LASHES	LIDS: no abnormalities / scaling / mucus / hyperaemia / redness / puss / oedema / frothy tear film / entropion / ectropion / pigmented lesions / thickening / other: EYELASHES: no abnormalities / ingrown / multiple / deposits / discharge / other:	LIDS: no abnormalities / scaling / mucus / hyperaemia / redness / puss / oedema / frothy tear film / entropion / ectropion / pigmented lesions / thickening / other: EYELASHES: no abnormalities / ingrown / multiple / deposits / discharge / other:
LID MARGIN *(normal: up to 6 glands obstructed with clear discharge)*	Lid margin: glands unobstructed / pus / oedema / frothy tear film / even / uneven / Meibomian glands obstruction / discharge / notched lid margin / other: MGD: 0 / 1 / 2 / 3 / 4	Lid margin: glands unobstructed / pus / oedema / frothy tear film / even / uneven / Meibomian glands obstruction / discharge / notched lid margin / other: MGD: 0 / 1 / 2 / 3 / 4
CORNEA AND LIMBUS	Clear / transparent / limbal vascularization / micro cists / vacuole / scars / oedema/ other: …… LIMBAL REDNESS: 0/1/2/3/4 LIMBAL VASCULARIZATION: 0/1/2/3/4	Clear / transparent / limbal vascularization / micro cists / ulcers / vacuole / scars / oedema/ other: …….. LIMBAL REDNESS: 0/1/2/3/4 LIMBAL VASCULARIZATION: 0/1/2/3/4
FBUT	.....[s] ….....[s] ….....[s]	.....[s] ….....[s] ….....[s]
FLOURESCEIN STAINING SCORE		
DED DIAGNOSIS	At least two out of the following: ☐ OSDI ≥ 25 ☐ Conjunctival staining score ≥ 2 ☐ Corneal staining score ≥ 2 ☐ FBUT ≤ 7 s	**NOTES:**

Appendix 5. Baseline visit evaluation sheet

Contact lenses to be removed and eye drops not used at least 3 days prior to evaluation

Subject code ☐☐☐☐ **Date:** ☐☐/☐☐/☐☐ **TIME:** ☐☐

At what time did the subject wake up? ☐☐ : ☐☐

Room Temperature: ☐☐.☐ **[°C/F]** **Relative Humidity:** ☐☐.☐ **[% RH]**

Number of blinks ☐☐ **/30s; Incomplete blinking noted? Yes / No;**

WHICH EYE (OD if SiHy was worn, OS if Hy was worn): ☐☐☐☐

DED Questionnaires: DEQ-5 score ☐☐ **OSDI** ☐ **Score** ☐ / ☐ / ☐

Answers ☐☐

TMH with OCULUS: ☐.☐☐ **[mm]**

Tear Osmolarity: ☐☐☐, ☐☐☐, ☐☐☐

M-NIKBUT 1) ☐☐ **2)** ☐☐. **3)** ☐☐

F-NIKBUT 1) ☐☐. **2)** ☐☐. **3)** ☐☐

Lipid Layer (video): ☐

Ocular Redness:	**Bulbar (Efron Scale):**		**Limbal (Efron Scale):**	
	Temporal	**Nasal**	**Temporal**	**Nasal**

SLIT LAMP EXAMINATION OD ☐ **(Use the slit lamp protocol)**

Fluorescein Tear Film Break-up Time 1) 2) 3) Fluo imaging ☐

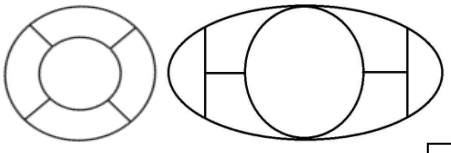
Corneal Thickness: ☐ **OCT Tear Meniscus Baseline Morphology:** ☐☐☐

Tear meniscus measurements for Tear Clearance Rate assessment:

☐**0s,** ☐**30s,** ☐**1min,** ☐**2 min,** ☐**3 min,** ☐**4 min,** ☐**5 min**

Ocular Staining (slit lamp protocol)

Fluo Image, FBUT: .....[s] ….....[s] ….....[s]



Conjunctival staining with LG ☐ **Lid wiper staining: Upper** ☐ **Lower** ☐

Meibomian gland imaging ☐ **Upper lid,** ☐ **Lower lid**

Appendix 6. Contact lens fit - evaluation sheet

Newly-fitted contact lens should be worn at least for 4 hours prior to afternoon visit

Subject code ☐ **Date: / / / Time**
Room Temperature [°C] Relative Humidity [%RH]

Instruction about CL'S usage and hygiene signed, explained and understood ☐
Contact Lens Fit (morning visit) - Px will be fit with SiHy in OD and hydrogel in OS

FITTED LENSES ☐ OD ☐ OS

AFTER 4 HOURS of CLS wear

Time:
Choose the CL based on the fit, subjective rating comfort, slit lamp, TFSQ assessment and vision.

	OD	OS
Fit:		
Centration:		
Horizontal Lag:		
On Blink		
PU Test		
Vision		

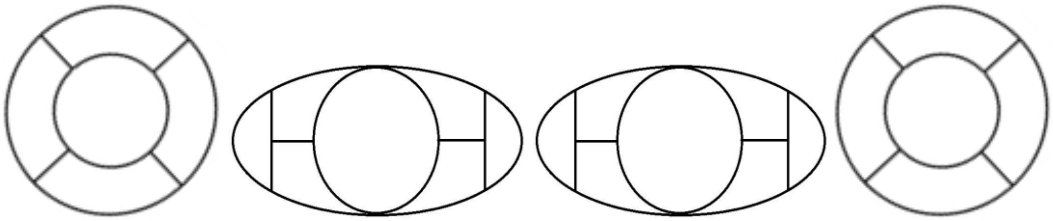
Better fit ☐ **Better comfort** ☐ **Better fit** ☐ **Better comfort** ☐

CONTACT LENS ASSESSMENT – ☐ **VIDEO OCULUS**
Assess and capture the subjective CL fit of the chosen lens
Primary gaze 3s. Temporally 3s, Nasally 3s
Blink in up gaze x3 waiting 3s after each; Push-up to mid cornea with lower lid and pull lid down as release x 3 waiting 5s after each.

NIKBUT Oculus

M-NIKBUT	**OD:**	**1)**	**.2)**	**3)**
	OS:	**1)**	**.2)**	**3)**
F-NIKBUT	**OD:**	**1)**	**.2)**	**3)**
	OS:	**1)**	**.2)**	**3)**

Ocular Staining (slit lamp)



OD FBUT: .....[s] ….....[s] ….....[s]
OS:FBUT:.....[s]….....[s].....[s]

Appendix 7. Instructions on contact lens care and wear

CONTACT LENS ADVICE

DO:

- Always wash your hand thoroughly before inserting, removing or handling your lenses and ensure hands are dry
- Remove lenses in the event of persistent irritation and contact us

DON'T:

- Sleep in your contact lenses,
- Lick your lenses or put them in your mouth,
- Use tissues or handkerchiefs to rub your lenses,
- Wear your lenses longer than advised,
- Wear your lenses if you think you may have an eye injury, infection or the lens might be damaged,
- Share your lenses with anyone else,
- Swim in your contact lenses,
- Wear for long plane journeys -they may dry out or you may want to sleep.
-

NEVER USE TAP WATER TO CLEAN YOUR LENSES!

Make-up advice:

- Apply make-up on after inserting contact lenses,
- Do not use mascara that flakes,
- When using hair spray, close your eyes to prevent it getting onto your lenses, or spray before inserting contact lenses,
- Do not share make-up tools with anyone and ensure they are not expired, as this could result in an infection.

With my signature I declare that I understand the abovementioned instructions and I am obliged to wear my newly-fitted contact lenses 5 days per week and not exceed 12 hours of daily contact lens wear

DATE:
Patients signature: ……..

(one signed copy for the subjects and one copy for the ESR)

Appendix 8. 2-week follow-up - evaluation sheet

Subject code Date: / / TIME

At what time did the patient woke up? ☐☐ **:** ☐☐

Room Temperature ☐ **°C** **Relative Humidity** ☐ **%RH**

DED Questionnaire:

OSDI ☐ **Score** ☐**/** ☐**/** ☐**,** **DEQ-5** ☐ **Score** ☐☐

SLIT LAMP CONTACT LENS SURFACE QUALITY ASSESSMENT

Observations: Clean/ Debris Yes / No /Oily / Protein deposit

Other artefacts:

AFTER LENS REMOVAL:

TEAR MENISCUS HEIGHT Oculus OD☐ OS☐

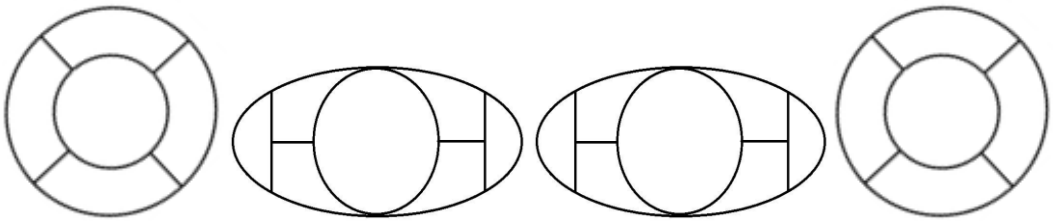
Lipid Layer thickness (video): OD☐ OS☐

Corneal Thickness OD☐ OS ☐

OCT: TEAR MENISCUS HEIGHT Baseline OS: ☐ ☐ ☐

Instil fluorescein - Ocular health examination (slit lamp)

Ocular Staining (slit lamp)



OD, FBUT: .....[s] ….....[s] ….....[s]

OS, FBUT: .....[s] ….....[s] ….....[s]

Observations:

Appendix 9. 3-month, 6-month and 12-month visit evaluation sheet

Subject code *Date:* *Time:* *Patient woke up at:* *and put the lenses on at:*

Room Temperature °C/F **Relative Humidity [%RH]**

OSDI ☐ **Answers:** ☐☐ **DEQ-5** ☐ **Score** ☐☐

WITH the contact lens on

NIKBUT Oculus

M-NIKBUT	OD:	1)	.2)	3)
	OS:	1)	.2)	3)
F-NIKBUT	OD:	1)	.2)	3)
	OS:	1)	.2)	3)

LENS REMOVAL

TMH with OCULUS: OD mm OS mm

Tear Osmolarity: OD 1) .2) 3)

OS 1) .2) 3)

Lipid Layer (video) OD ☐ OS ☐

OCT CORNEAL TICKNESS: OD☐ OS ☐

OCT: TMH 3x☐ 0s ☐30s☐1min☐2 min ☐3 min

Ocular Redness OD:

Bulbar (Efron Scale): Limbal (Efron Scale):

Temporal Nasal Temporal Nasa

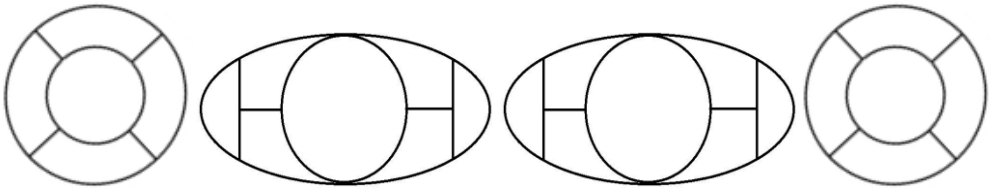
Ocular Redness OD:

Bulbar (Efron Scale): Limbal (Efron Scale):

Temporal Nasal Temporal Nasal

Ocular Staining (slit lamp)

OD + Fluo Image, FBUT: .....[s] ….....[s] ….....[s]



OS + Fluo Image, FBUT: .....[s] ….....[s] ….....[s]

Conjunctival staining with LG (primary gaze) ☐

Lid wiper staining: Upper Marx's line ☐ Lower Marx's line☐

Meibomian gland imaging ☐ Upper lid, ☐ Lower lid

Appendix 10. Control visit evaluation sheet

Contact lenses to be removed and eye drops not used at least 3 days prior to visit

Subject code Date: TIME: At what time did the subject wake up?
Room Temperature: [°C/F] Relative Humidity: [% RH]
Incomplete blinking noted? Yes / No;

WHICH EYE (OD if SiHy was worn, OS if Hy was worn): ☐

DED Questionnaires: DEQ-5 Score ☐☐ OSDI Answers ☐☐

TMH with OCULUS: ☐.☐☐ [mm]

Tear Osmolarity: ☐☐☐,☐☐☐,☐☐☐

M-NIKBUT **1)** **.2)** **3)**

F-NIKBUT **1)** **.2)** **3)**

Lipid Layer (video): ☐

Ocular Redness: Bulbar (Efron Scale): Limbal (Efron Scale):
Temporal Nasal Temporal Nasal

SLIT LAMP EXAMINATION OD ☐ (Use the slit lamp protocol)
Fluorescein Tear Film Break-up Time 1) 2) 3)

Fluo imaging OCULUS: ☐

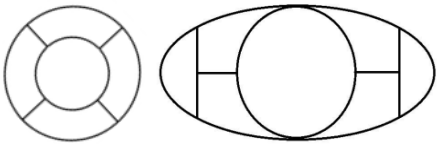
Corneal Thickness: ☐

OCT Tear Meniscus Baseline Morphology: ☐☐☐

Tear meniscus measurements for Tear Clearance Rate assessment

Ocular Staining (slit lamp protocol)

Fluo Image, FBUT: .....[s] ….....[s] ….....[s]



Conjunctival staining with LG (primary gaze) ☐

Lid wiper staining: Upper Marx's line ☐ Lower Marx's line ☐

Meibomian gland imaging: Upper lid, ☐ Lower lid

SCIENTIFIC CONTRIBUTION RELATED TO THE THESIS

JOURNAL ARTICLES

J01: Garaszczuk IK, Iskander DR. Qualitative assessment of tear dynamics with fluorescein profilometry, *Contact Lens and Anterior Eye* 2017; 40 (4) 208-212

J02: Mousavi M, Jesus, DA, **Garaszczuk, IK**, Szczesna-Iskander, DH, Iskander, DR. The utility of measuring tear film break-up time for prescribing contact lenses. *Contact Lens and Anterior Eye* 2018; 41 (1), 105-109

J03: Garaszczuk IK, Mousavi M, Iskander, DR, Montés-Micó R, Cerviño Expósito A, (2018), Tear clearance rate assessment with optical coherence tomography. *Contact Lens and Anterior Eye* 2018; 41, S78-S79

J04: Garaszczuk IK, Iskander DR, Nowa metoda pomiaru parametru wymiany filmu łzowego przy pomocy profilometru fluoresceinowego, (A new method of tear turnover rate assessment with fluorescein profilometry) *Optyka* 2017; 5/2017, [in Polish]

J05: Garaszczuk, IK, Montes Mico, R, Iskander, DR, Cerviño Expósito A. The tear turnover and tear clearance tests–a review. *Expert review of medical devices* 2018; 15 (3), 219-229

J06: Mousavi M, **Garaszczuk IK**, Jesus DA, Szczesna-Iskander DH, Armstrong RA, Iskander DR. Ocular physiology during the period of 12 months of modern daily disposable soft contact lens wear. *PLOS One* **[Under Revision]**

J07: Garaszczuk IK, Mousavi M, Szczesna-Iskander DH, Iskander, DR, Cerviño Expósito A. A 12-month prospective study of tear osmolarity in contact lens wearers fitted with daily disposable soft contact lenses. *PLOS One* **[Ready for submission]**

J08: Garaszczuk IK, Mousavi M, Cerviño Expósito A, Iskander DR. Prospective longitudinal study of the central lower tear meniscus morphology in contact lens wearers refitted with daily disposable soft contact lenses. *Optometry and Vision Science* **[Ready for submission]**

Citation count: 5

COMMUNICATIONS IN CONGRESSES

Poster presentations

C01: Garaszczuk IK, Iskander DR, Montés Micó R, Evaluación de la tasa de aclaramiento de la película lagrimal mediante un perfilómetro corneo-escleral de Fourier, 24 *Congreso Internacional de Optometria, Contactología y Óptica Oftálmica* 2016. 8-10 April 2016, Madrid, Spain.

C02: Garaszczuk IK, Iskander DR, Tear dynamics evaluation with fluorescein profilometry and optical coherence tomography*, 8th International conference on the Tear Film and Ocular Surface* Society, 8- 10 September 2016, Montpellier, France.

C03: Garaszczuk IK, Mousavi M, Cerviño Expósito A, Szczesna-Iskander DH, Iskander DR, Jesus DA, Scleral radius estimation based on anterior eye surface. *The Annual Meeting of the Association for Research in Vision and Ophthalmology* (ARVO) 2017. 7-11 May 2017, Baltimore, United States.

C04: Jesus DA, **Garaszczuk IK**, Mousavi M, Iskander DR, Influence of IOP fluctuation on corneal micro-structure. *The Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO)* 2017. 7-11 May 2017, Baltimore, United States.

C05: Mousavi M, **Garaszczuk IK**, Jesus DA, Szczesna-Iskander DH, Iskander DR. The impact of daily disposable soft contact lens wear on tear film surface quality over a three month period. *European Association for Vision and Eye Research Conference*, 27-30 September 2017, Nice, France

C06: Mousavi M, **Garaszczuk IK**, Szczesna-Iskander DH, Iskander DR. Changes in tear film physiology during soft contact lens wear, *The Annual Meeting of the Association for Research in Vision and Ophthalmology* (ARVO) 2018, 29 April - 3 May 2018, Honolulu, United States

C07: Garaszczuk IK, Mousavi M, Montés Micó R, Cerviño Expósito A, Iskander DR. Changes in the lower tear meniscus morphology during contact lens wear. *The Annual Meeting of the Association for Research in Vision and Ophthalmology* (ARVO) 2018, 29 April - 3 May 2018, Honolulu, United States

Oral presentations

O01: Lafosse E, Ferrer-Blasco T, Esteve Taboada JJ, **Garaszczuk IK**, García-Lázaro S. Tecnicas no invasivas para la evaluación del menisco lagrimal, 24 *Congreso Internacional de Optometria, Contactología y Óptica Oftálmica (OPTOM2016)*, 2016, 8-10 April 2016, Madrid, Spain.

O02: Mousavi M, **Garaszczuk IK**, Jesus DA, Szczesna-Iskander DH, Iskander DR, Relative performance of well fitted hydrogel and silicone hydrogel contact lenses, *British Contact Lens Association (BCLA) Clinical Conference* 2017, Liverpool, UK

O03: Garaszczuk IK, Mousavi M, Iskander DR, Montés Micó R, Cerviño Expósito A, Tear Clearance Rate assessment with optical coherence tomography, *British Contact Lens Association Clinical Conference* 2017, Liverpool, UK.

O04: Garaszczuk IK, My year-long journey as a vision science researcher. Invited Speaker, *CooperVision's Future Ocular Research Creativity Event (FORCE)* 2017, Budapest, Hungary.

OTHER SCIENTIFIC COMMUNICATIONS

OC01: Garaszczuk IK, Iskander DR, Nowa metoda pomiaru parametru wymiany filmu łzowego przy pomocy profilometru fluoresceinowego, Optyka 5/2017 **[Article in professional press]**

OC02: Garaszczuk IK, Konferencja naukowa Tear Film and Ocular Surface 2016 - relacja, *Optyka* 5/2016, 36-37 [**Article in professional press]**

OC03: Garaszczuk IK, 40. Konferencja kliniczna BCLA 2017 – relacja, Optyka 4/2017, 30-31 **[Article in professional press]**

R01: Reviewer for *Contact Lens and Anterior Eye*, 2017

AWARDS AND GRANTS

A01: Polish National Championship in CooperVision's Future Ocular Research Creativity Event (FORCE), research title: *Assessing Tear Clearance Rate utilizing a corneo-scleral profilometer and optical coherence tomography*, Wroclaw, Poland, 1 March 2016

A02: International championship in CooperVision's Future Ocular Research Creativity Event (FORCE). European Student of the Year 2016, 16 April 2016, Budapest, Hungary

G01: European Dry Eye Network (EDEN) project funded by European Union's Horizon2020 Research and Innovation Programme under Marie Skłodowska Curie Grant agreement number 642760.