

RESEARCH

Title: Inter-week variation of meibometry and break-up time in healthy subjects

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Running title: Inter-week variation of meibometry and BUT

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Clinical relevance: **Variation with time** in the tear film parameters should be considered by the clinician, since the time when measurements are made can influence in a proper diagnosis.

Background: A hallmark of dry eye is an unstable tear film associated with variability in objective daily measures. The purpose of the present study was to evaluate the inter-week repeatability of meibometry, Break-up Time (BUT) and Maximum Blink Interval (MBI) in healthy subjects.

Methods: Forty healthy subjects were recruited for the study. Meibometry, BUT and MBI were performed two times in two sessions one-week apart. Meibum from the lower eyelid was collected and quantified with the Meibometer® MB550. Five meibomian curves were generated by tape and each peak value was averaged as a mean value per session. BUT and MBI were performed after fluorescein instillation three times recorded by a DV-3 camera attached to the slit-lamp. BUT and MBI videos were analysed by a second masked observer who quantified their values in frames. Both BUT and MBI values were calculated by averaging only the two most similar measurements of the three evaluated.

Results: No statistical difference between inter-week sessions was obtained for the meibometry ($p > 0.340$; Wilcoxon test), BUT ($p > 0.326$; Wilcoxon test) and MBI values ($p \geq 0.248$; Wilcoxon test). Inter-week differences were low **for** BUT and MBI when times analysed were no longer than 15 seconds (both $p \geq 0.586$; Wilcoxon test). There was found a correlation between BUT and MBI ($r \geq 0.668$, $p < 0.001$; Spearman Rho), while a no **statistical** correlation was obtained between Meibometry results and BUT or MBI (all $p \geq 0.194$; Spearman Rho).

Conclusion: The present study showed that meibum secretion **and** the tear film stability present **good** intersession repeatability, and are stable **along with** sessions in healthy patients.

Many tests have been developed to examine different aspects of the ocular surface, such as **the** physiology of the tear film or corneal and conjunctival integrity. The tear film lipid layer is principally compounded by meibomian glands lipid secretion.¹⁻³ This lipid secretion is a clear liquid fluid called meibum, secreted over the lower and upper eyelid margins, composed by a mixture of cholesterol, wax, hydrocarbons, and phospholipids. Meibum is spread over the ocular surface forming the Tear film lipid layer, which performs an important role in reducing the aqueous layer evaporation.¹⁻³ Tear film stability plays an important role in the diagnosis and management the **dry eye disease**. It can be measured by many different techniques, being the most popular the Break-Up Time (BUT) test with fluorescein instillation into the ocular surface.^{4,5}

Additionally to this test, the Maximum Blink Interval (MBI) parameter, defined as the time that subjects are able to keep the eyes open, has also been proposed as an indicator of tear film quality.⁵⁻⁷ Abnormal production of the meibomian glands lipid secretion could also lead to a tear film instability, and therefore, to an alteration of the **BUT or the MBI tests**. In this sense, meibum secretion can be quantified by the meibometry technique,^{1,2,8-13} so evaluation of the tear film stability and meibomian gland secretion can play an important role in the early diagnosis of the dry eye disease. The most prevalent dry eye type is originated by the meibomian gland dysfunction,¹⁴ being defined by Tear Film & Ocular Surface Society as “a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. It may result in alteration of the tear film, symptoms of the eye irritation, clinically apparent inflammation, and ocular surface disease.”¹⁵

Ocular surface parameters and tear film characteristics could oscillate due to the environmental influence, such as humidity and temperature, or physiological variations.^{3,6,16,17} The possibility of the **variations over time** in the tear film parameters should be considered by the clinician, since the time when tear film measurements are made can be critical for the proper diagnosis. A hallmark of dry eye is an unstable tear film, which is associated with variability in objective measures of signs and symptoms on this disease.^{16,18} Indeed, the variability of some parameters has been recommended as a feature that clinicians should specifically be looking on diagnosis as a representation of the loss of tear film homeostasis that occurs during the disease.^{19,20}

Because of the enormous interest in the evaluation of the functionality and integrity of the meibomian glands, a wide number of tests and instruments have been proposed such as the Meibomian Gland Expression evaluation, Meiboscopy, Meibography or

Confocal microscopy.^{3,15,21,22} However, most clinical tests based on determining tear quality and quantity show high variability. This determines that clinicians recommend a large number of tests and currently there is no gold standard diagnostic method. Therefore, there is always a need to develop and study new diagnostic methods. Meibometry allows a rapid and in situ evaluation of meibomian gland secretion.^{2,8,10-12,23} This technique is little used in the general clinic because it is not standardized and more investigation is necessary as has been proposed by The International Workshop on Meibomian Gland Dysfunction.³ To our knowledge, there are no studies about meibometry and BUT by video-analysis technique⁷ inter-week repeatability done in the same population. Therefore, the present study aimed to analyse the inter-week repeatability of meibometry, BUT and the MBI in healthy subjects.

METHODS

Sample size calculation and recruitment

For the sample size calculation, PS Power and Sample Size Calculations Version 3.1.2 (Copyright © by William D. Dupont and Walton D. Plummer) was used. The study was planned as a continuous dependent response variable from paired measurements of subjects. The sample size was estimated about the meibum secretion (meibometry).^{1,2,8-13} The literature review showed that meibometry mean standard deviation (SD) of repeated measures is normally distributed with a mean value of 133,8 Meibometer units and a difference in the mean response of matched pairs is 63 Meibometer units.^{2,9,13,23} So, to be able to reject the null hypothesis that this response difference is zero with probability (power) of 0.80, a minimum of 37 pairs of subjects were needed to study; the Type I error probability associated with this test was 0.05. To accomplish a more reliable study, a larger population was recruited.

A total of 40 volunteer subjects (28 women and 12 men) of mean age 21.7 ± 4.53 (from 18 to 40 years) were recruited from subjects attending to the Optometry Clinic of the Optometry Faculty (USC). Since the aim of the study was to evaluate the inter-session repeatability of the studied test, only healthy subjects were recruited and scheduled for the study; therefore, following DEWS II report,^{16,19} during recruitment process subjects excluded if they failed to fulfil more than one of these criteria: a score < 13 for Surface Disease Index (OSDI) questionnaire,^{16,24} tear film osmolarity < 316 mOsm/l,^{16,25} corneal staining grade ≤ 1 on the Oxford Grading Scale,^{16,26} and a central tear meniscus height without fluorescein $\geq 0.20\text{mm}$ ^{16,27} (Table 1 shows ocular surface demographics characteristic of subjects who participated in this study). Subjects were also excluded if

they had a history of a conjunctival, scleral or corneal disease, an active ocular disease (including dry eye disease and meibomian gland dysfunction) or ocular allergy, prior eye surgery (including refractive surgery or eyelid tattooing), glaucoma, diabetes mellitus, thyroid disorders, were pregnant or breast-feeding, wore contact lenses or had a systemic inflammatory/autoimmune disease. No participant was under any type of medication or used artificial tears at the time of the testing session. All patients gave their written informed consent to be included in the study. The study protocol followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Santiago de Compostela.

Study design

The qualified subjects were scheduled for measurements of meibometry, BUT and MBI two times in two sessions with an interval of one week between them. In order to minimize the possible influence of fluorescein over the meibomian gland secretion, meibometry and BUT were always obtained following the same sequence; In each session, meibometry was always obtained first, and 10 to 15 minutes later, the BUT was recorded after fluorescein instillation. To avoid the effects of overstating the precision of statistical estimates, only the right eye was examined.²⁸ To minimize the possible diurnal variation all the study sessions were made at the same time of day (between 4.00 and 6.00 pm), while environmental conditions of the clinic were always controlled and maintained under similar conditions of temperature (20-23°C) and humidity (50-60%) to avoid any influence in the meibomian gland delivery or tear film stability.^{20,23,29-31}

Meibometry

The meibomian secretion value was obtained by using the Meibometer® MB550 (Courage-Khazaka electronic GmbH, Cologne, Germany).^{8,9,11-13} This device is based on a photometer unit, which was connected to a computer in order to show the recorded data in tables and curve graphs. To collect a meibomian dropout, a matt synthetic tape was used. A 17 cm length marked at 3 cm and 6 cm portion of the tape was cut to make a loop with always the same shape. The lower eyelid was everted, tape's loop got in contact in the central portion for 10 seconds and the sample collected. A minute was taken to let the meibomian secretion dried in the tape, and then the loop was introduced in the instrument. Following previous reports and pretending to reduce possible variability per session,^{9,11} a total of five meibomian curves were generated by analysing each tape five times by the photometer. Data were

provided in Meibometer units,^{8,9,11,12} Once the curves were obtained, the highest value of each curve was taken, and the mean was calculated for each patient per session.^{9,11}

Break-up time and Maximum blink interval calculation

A volume of 2 µl of non-preserved 2% sodium fluorescein was instilled on the inferotemporal bulbar conjunctiva with a micropipette. The patient was requested to place on the chinrest of Topcon slit-lamp and the ocular surface was examined with 16x magnification, Wratten 12 yellow filter, and cobalt blue light.³² **Room light**

conditions were always kept constant in order to not generate differences between video recordings and possible influence in the evaluation of the test. Patients were instructed to blink 3 times **and then** keep the eyes open for as long as possible.⁵⁻⁷

During all the **processes**, the tear film stability was recorded by a DV-3 camera attached to the Topcon slit-lamp connected to a computer. Stability recording was always performed 3 times per patient and per session by the same observer.

From each video, two parameters were extracted: 1) the tear film BUT, defined as the time calculated between **the last blink and the first black** spot or line,^{4,7} and 2) the MBI, defined as the maximum time that a patient was able to keep his eyes opened after the last blink.⁵⁻⁷ Video recordings were analysed later by a second masked observer who counted the frames for each value with the software VirtualDub. These frames were converted into seconds (15 frames per second). As was previously recommended to reduce variability, BUT and MBI values were calculated by averaging only the two most similar measurements of the three evaluated.^{4,7}

Statistical analysis

IBM SPSS Statistics v.23 software (SPSS Inc., Chicago, IL) was used for the data analysis. Significance was set at $p \leq 0.05$ for all tests.

Before any analysis, the normal distribution of the data was checked using the Shaphiro-Wilk test. Meibometry, BUT and MBI did not follow a normal distribution (all $p \leq 0.002$). Therefore, non-parametric descriptive statistics were showed (median, inter-quartile range (IQR)). However, mean and standard deviation (SD), as well as maximum and minimum values, were also provided to enable comparisons with other studies.

For all parameters, a non-parametric Wilcoxon test was used to analyse the differences between the first and the second session.³³ Also, plots of Bland-Altman were used to examine repeatability across the entire range measured. This method describes the similarity between both sessions by representing mean versus differences. Therefore,

the mean of the difference and the 95% limits of repeatability (mean difference \pm 1.96 x standard deviation) were also calculated.

The relationship between the variables tested was assessed through the Spearman Rho correlation for non-parametric data.

RESULTS

Descriptive statistics of each session and test (meibometry, BUT and MBI) are shown in Table 2 (n = 40 each session).

Inter-week repeatability of meibometry

Differences calculated by a Wilcoxon test between the meibometry values obtained in each session showed no significant difference between sessions (p = 0.340, Table 3). These results are graphically represented by a Bland and Altman plot in Figure 1 (Mean vs. differences between sessions).

Inter-week repeatability of BUT and MBI

Differences between the results of BUT and MBI obtained in each session were calculated. Similar to meibometry, the Wilcoxon test showed no significant difference between sessions on both BUT and MBI measurements (both p \geq 0.248, Table 3) indicating no inter-week variation in both tests. Figures 2 and 3 shows the Bland-Altman plot of means versus differences between sessions for BUT and MBI results, respectively. MBI showed a higher bias range than BUT.

Figures 2 and 3 show that bias was higher for BUT measurements greater than 15 seconds, therefore a higher difference between means and differences calculated between the values obtained in each session. Based on these results and according to previous reports,^{4,7} the BUT and MBI variability between inter-week sessions was re-analysed by considering only a mean \leq 15 seconds (Table 4). Similarly, on the total sample analysis, there was no difference in the BUT results between sessions (Wilcoxon test p = 0.586, Table 4) as well as in the MBI results between sessions (Wilcoxon test p = 0.733, Table 4). Furthermore, in both cases, the mean difference between measurements performed on each session (close to 0) and bias according to the 95% confidence interval was remarkably lower (Table 3, Figures 2 and 3).

Correlation between parameters

There was found a positive correlation between BUT and MBI parameter both in the first (Spearman Rho Correlation; r = 0.668, p < 0.001) and the second session (Spearman Rho Correlation; r = 0.848, p < 0.001). However, it was not found a statistically correlation between Meibometry results and BUT or MBI, neither in the first

or the second session (Spearman Rho Correlation; Meibometry vs. BUT, all $p \geq 0.202$; Meibometry vs. MBI, all $p \geq 0.194$).

DISCUSSION

Variations over time in tear film variables have not been clearly established yet. The present study analyses the inter-week repeatability of meibometry, BUT and MBI. It was found that none of these three tests showed variation in their results between the values recorded in two sessions (one week apart) on healthy subjects.

Dry eye disease and meibomian gland dysfunction greatly reduce the quality of life of the patient, therefore early diagnosis and management should be performed in order to improve the health of the patient.^{16,34} The homeostasis loss triggered by an unstable tear film generates many dry eye symptoms; hence tests like meibometry (meibum secretion) and BUT/MBI are important for categorizing the tear film stability.

Meibometry measures the quantity of meibum secreted by the meibomian gland, which is the main component of the tear film lipid layer.^{3,14} The lack of tear film lipid layer generates an unstable tear film leading to low or high variability in BUT values. Both techniques performed together give important information about tear film status and help in the categorization of meibomian gland dysfunction.^{3,14,16} Commonly, meibum secretion and stability studies have focused only on the diagnosis performance of the test. In addition to the cut-off limitation or differences between measurements, **the variations with time** of those parameters should be assessed and established in order to minimize possible diagnosis misleading.

Due to the multifactorial aetiology of the dry eye or the MGD, its diagnosis is extremely difficult. This determines that clinicians recommend a large number of tests and currently there is no gold standard diagnostic method. Many tests have been proposed in order to evaluate the functionality and integrity of the meibomian glands like the Meibomian Gland Expression evaluation, Meiboscopy, Meibography, Confocal microscopy or Meibometry.^{2,3,8,10-12,15,21-23} However, there is no consensus on the diagnostic gold standard, so there is always a need to develop and study new diagnostic methods. It has been hypostatized that the lipid secretion of the meibomian glands (meibum) is the main composition of the tear film lipid layer and has an important role in reducing the aqueous layer evaporation.³ In order to measure that secretion, Chew et al.¹⁰ implemented the meibometry technique, **oriented to evaluate the amount of meibomian lipids. The device used for meibometry is a modification** from an instrument used for measure skin lipids, Sebumeter, which was formed by two

parts; first, a photometer and second, a measuring head with a plastic loop where the meibum was collected. The device uses photometry to quantify the casual level or amount of lipids on the lid margins in the interblink period.^{2,8,10,12,23} At present, there are few studies that addressed the Meibometer,^{2,10,23} and not all were in humans.^{8,12}

Regarding the variation with time of this technique, other Chew et al.¹ report found no significant difference in meibometry results between morning (10.30 h – 13.30 h) and afternoon (16.30 h – 18.30 h) on non-dry subjects. Those results are in concordance with the present study where no variation between inter-week sessions was found so it seems that meibometry measurements are timely stable both daily and weekly on healthy subjects. Despite the stability between sessions, they found age and gender variations: lowest values of meibometry were obtained in children under 14 years old and the highest values were reached in the 60-69 years old for males and 70-79 years old for females. Yokoi et al.² showed that meibometry values were significantly lower in meibomian gland dysfunction patients than both other dry eye disease patients and control subjects. Time variability, gender, age, between observers and inter-eye variations have their importance in dry eye disease and meibomian gland dysfunction diagnosis. To avoid more possible variations in our study, temperature maintenance on the laboratory was necessary because previous meibometry studies have shown that external heating or cooling affect meibomian gland delivery by its effect on the viscosity and flow characteristics of the oil.²³ Because of the many forms of presentation of the dry eye, it is expected that will not exist inter-week repeatability in the meibometry values; this issue will need future investigation.

Other authors studied BUT repeatability, Cox et al.³⁵ measured the BUT (slit-lamp) and Non-invasive BUT (Keratograph 4 instrument) in two sessions separated by 7 (\pm 2) days. While they concluded that Non-invasive BUT had a better agreement between sessions than BUT, in concordance with the present study, there was found no inter-week variation in the BUT value. Besides, they also observed better agreement between sessions for lower measures, similar to the present study when cut-off values of ≤ 15 seconds were implemented. Fernández et al.³⁶ studied BUT repeatability using two different technics, Non-invasive BUT measured by videokeratoscope and with Optical Quality Analysis System, showing that No-Invasive BUT values cannot be used interchangeably but there was good intersession repeatability in both techniques. On the other hand, there is some controversy in how the tear film stability value changes over time in both healthy and pathological subjects.^{7,29,31,37} Patel et al.²⁹ studied precorneal tear film stability and how it oscillates among a 12-hour period, they

concluded that precorneal tear film stability varies through all day. This conclusion is based on the fact that after waking the mucin levels are lower than on the rest of the day; so, Patel observed that if he excluded the measure after awakening, BUT does not vary. Moreover, Lira et al.³⁰ reported a decrease in BUT values in 21 healthy subjects between the afternoon (5 - 6 pm) compared to the morning (9 - 10 am). Similarly, Bitton et al.³¹ observed NIBUT differences during the day in dry eye patients. Contrary to pathological subjects, the same study of Bitton et al.³¹, and a study of Walker et al.³⁷ reported no variation between these two points of the day on NIBUT values of 15 healthy subjects, or in BUT values of dry eye subjects. Puihas et al.²⁰ studied the changes during the day in the protein concentration and tear film stability of 9 healthy subjects. They observed that the tear film stability was higher in the morning than in the afternoon, according to the superior value of surface tension obtained. For protein concentration, immunoglobulin A showed significant statistical differences between the morning and the afternoon decreasing its concentration, tumour necrosis factor α also decreased but not statistically significant. Authors like Begley et al.⁵ studied the tear break-up dynamics, which involves BUT and the area of film break-up. They observed that BUT tends to be shorter in dry eye patients than in controls, but the difference was not significant which could be influenced by the small sample (10 controls and 10 dry eye subjects). Nevertheless, their measure of the area of film break-up, defined as the dark area observed in the BUT before the MBI, showed that these areas were bigger (biggest extension), and formed more rapidly, in dry eye patients than in controls. Wang et al.³⁸ studied the influence of incomplete blinking on the ocular surface. They showed that patients who have incomplete blinking have worse Non-invasive BUT and poorer tear film lipid layer. Likewise, incomplete blinking could predispose patients to an evaporative dry eye caused by greater levels of meibum not distributed over the ocular surface resulting in low Non-invasive BUT. Overall, these studies showed that BUT could vary by many parameters.^{7,29,31,37,38} In a previous report,⁷ we found that there were no daily variations in healthy patients, similar to the present study where no week-variation between sessions was found in the same type of sample. Both the variation with time analysis studies, daily and weekly, should be performed in a pathological group sample.

The MBI parameter⁵⁻⁷ adds information to that offered by BUT because it indicates when tear film break-up causes discomfort; it has been shown to be longer in controls than in patients with dry eye.⁵ The mean/median MBI values obtained in the present study (Tables 2 and 4) were similar to that obtained by Inomata et al.³⁹ and in one of

our previous report,⁷ but lower than those reported by Liu et al.⁶ and Begley et al.⁵ This discrepancy could reflect the high variation in MBI observed both in controls and dry eye subjects.⁵⁻⁷ As previous,^{5,7,40} it was found a strong correlation between BUT and MBI (Spearman Rho Correlation; $r = 0.668$, $p < 0.001$). Similar to the BUT, MBI showed no variation between session (Tables 3 and 4), and a positive correlation with the Meibometry results. Inomata et al.³⁹ studied a total of 294 subjects (194 with dry eye and 98 without dry eye) and observed that MBI values were significantly shortened in the dry eye group compared to the non- dry eye group. They concluded that MBI is a useful technique for the screening of dry eye although MBI values could not distinguish between evaporative or obstructive dry eye.

The strength of this study lies in the fact that the ambient temperature remained stable during the evaluation, while additionally all of the measurements were performed at the same point of the day, further reducing any potential variation in values. In addition, the screening of the sample was exhaustive and supported by the literature recommendations,^{16,19,24-27} in order to focus only on healthy patients and avoid any interference of pathological subjects. A limitation of the study was that the technique only takes a sample of one-third of the free eyelid margin, as known, not all the sebaceous glands secrete at the same time and this could influence the measure of the meibum.³ The Meibometer shows other limitations, such as one example reported by Benz et al.⁸, who used the Meibometer to perform the technique in the lower eyelids of dogs and found a significant difference in right eye measures between examiners, which could make questionable the precision of the instrument. Future studies might focus on analyzing differences in meibometry values, BUT and MBI between groups of dry eye and non- dry eye disease patients also could analyse different dry eye subgroups like evaporative dry eye or dry eye disease caused by meibomian gland dysfunction. Repeatability of meibometry in dry eye disease groups should be studied and its relationship, if it exists, with other techniques for meibomian gland evaluation like meibography. As mentioned in the limitations of the study, not all sebaceous glands secrete at the same time so future studies should analyse how to change the meibometry values along the day, implementing the measures always in the same zone of the free eyelid margin.

In summary, our findings indicate that meibomium secretion measured by meibometry, as well as the tear film stability, present good intersession repeatability and are stable along **with** sessions in healthy patients.

REFERENCES

- 1 Chew CK, Hykin PG, Jansweijer C et al. The casual level of meibomian lipids in humans. *Curr Eye Res* 1993; 12: 255-259.
- 2 Yokoi N, Mossa F, Tiffany JM et al. Assessment of meibomian gland function in dry eye using meibometry. *Arch Ophthalmol* 1999; 117: 723-729.
- 3 Tomlinson A, Bron AJ, Korb DR et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Invest Ophthalmol Vis Sci* 2011; 52: 2006-2049.
- 4 Cho P, Brown B. Review of the tear break-up time and a closer look at the tear break-up time of Hong Kong Chinese. *Optom Vis Sci* 1993; 70: 30-38.
- 5 Begley CG, Himebaugh N, Renner D et al. Tear breakup dynamics: a technique for quantifying tear film instability. *Optom Vis Sci* 2006; 83: 15-21.
- 6 Liu H, Begley CG, Chalmers R et al. Temporal progression and spatial repeatability of tear breakup. *Optom Vis Sci* 2006; 83: 723-730.
- 7 Pena-Verdeal H, Garcia-Resua C, Ramos L et al. Diurnal variations in tear film break-up time determined in healthy subjects by software-assisted interpretation of tear film video recordings. *Clin Exp Optom* 2016; 99: 142-148.
- 8 Benz P, Tichy A, Nell B. Review of the measuring precision of the new Meibometer MB550 through repeated measurements in dogs. *Vet Ophthalmol* 2008; 11: 368-374.
- 9 Garcia-Resua C, Pena-Verdeal H, Giraldez MJ et al. Clinical relationship of meibometry with ocular symptoms and tear film stability. *Cont Lens Anterior Eye* 2017; 40: 408-416.
- 10 Chew CK, Jansweijer C, Tiffany JM et al. An instrument for quantifying meibomian lipid on the lid margin: the Meibometer. *Curr Eye Res* 1993; 12: 247-254.
- 11 Sebbag L, Kass PH, Maggs DJ. Reference values, intertest correlations, and test-retest repeatability of selected tear film tests in healthy cats. *J Am Vet Med Assoc* 2015; 246: 426-435.
- 12 Ofri R, Orgad K, Kass PH et al. Canine meibometry: establishing baseline values for meibomian gland secretions in dogs. *Vet J* 2007; 174: 536-540.
- 13 Nakayama N, Kawashima M, Kaido M et al. Analysis of Meibum Before and After Intraductal Meibomian Gland Probing in Eyes With Obstructive Meibomian Gland Dysfunction. *Cornea* 2015; 34: 1206-1208.

- 14 Craig JP, Nichols KK, Akpek EK et al. TFOS DEWS II Definition and Classification Report. *Ocul Surf* 2017; 15: 276-283.
- 15 Nelson JD, Shimazaki J, Benitez-del-Castillo JM et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci* 2011; 52: 1930-1937.
- 16 Wolffsohn JS, Arita R, Chalmers R et al. TFOS DEWS II Diagnostic Methodology report. *Ocul Surf* 2017; 15: 539-574.
- 17 Li M, Du C, Zhu D et al. Daytime variations of tear osmolarity and tear meniscus volume. *Eye Contact Lens* 2012; 38: 282-287.
- 18 Lemp MA, Bron AJ, Baudouin C et al. Tear osmolarity in the diagnosis and management of dry eye disease. *Am J Ophthalmol* 2011; 151: 792-798 e791.
- 19 Sullivan B. Challenges in using signs and symptoms to evaluate new biomarkers of dry eye disease. *Ocul Surf* 2014; 12: 2-9.
- 20 Puihas A, Sampaio P, Castanheira EM et al. Comparison of IgA, TNF-alpha and surface tension of the tear film in two different times of the day. *Cont Lens Anterior Eye* 2013; 36: 140-145.
- 21 Pflugfelder SC, Tseng SC, Sanabria O et al. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. *Cornea* 1998; 17: 38-56.
- 22 Matsumoto Y, Sato EA, Ibrahim OM et al. The application of in vivo laser confocal microscopy to the diagnosis and evaluation of meibomian gland dysfunction. *Mol Vis* 2008; 14: 1263-1271.
- 23 Nagymihalyi A, Dikstein S, Tiffany JM. The influence of eyelid temperature on the delivery of meibomian oil. *Exp Eye Res* 2004; 78: 367-370.
- 24 Miller KL, Walt JG, Mink DR et al. Minimal clinically important difference for the ocular surface disease index. *Arch Ophthalmol* 2010; 128: 94-101.
- 25 Stahl U, Willcox M, Stapleton F. Osmolality and tear film dynamics. *Clin Exp Optom* 2012; 95: 3-11.
- 26 Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea* 2003; 22: 640-650.
- 27 Garcia-Resua C, Santodomingo-Rubido J, Lira M et al. Clinical assessment of the lower tear meniscus height. *Ophthalmic Physiol Opt* 2009; 29: 487-496.
- 28 Ray WA, O'Day DM. Statistical analysis of multi-eye data in ophthalmic research. *Invest Ophthalmol Vis Sci* 1985; 26: 1186-1188.

- 29 Patel S, Bevan R, Farrell JC. Diurnal variation in precorneal tear film stability. *Am J Optom Physiol Opt* 1988; 65: 151-154.
- 30 Lira M, Oliveira ME, Franco S. Comparison of the tear film clinical parameters at two different times of the day. *Clin Exp Optom* 2011; 94: 557-562.
- 31 Bitton E, Keech A, Jones L et al. Subjective and objective variation of the tear film pre- and post-sleep. *Optom Vis Sci* 2008; 85: 740-749.
- 32 Johnson ME, Murphy PJ. The Effect of instilled fluorescein solution volume on the values and repeatability of TBUT measurements. *Cornea* 2005; 24: 811-817.
- 33 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1: 307-310.
- 34 Craig JP, Nelson JD, Azar DT et al. TFOS DEWS II Report Executive Summary. *Ocul Surf* 2017; 15: 802-812.
- 35 Cox SM, Nichols KK, Nichols JJ. Agreement between Automated and Traditional Measures of Tear Film Breakup. *Optom Vis Sci* 2015; 92: e257-263.
- 36 Fernandez J, Rodriguez-Vallejo M, Martinez J et al. Agreement and repeatability of objective systems for assessment of the tear film. *Graefes Arch Clin Exp Ophthalmol* 2018; 256: 1535-1541.
- 37 Walker PM, Lane KJ, Ousler GW, 3rd et al. Diurnal variation of visual function and the signs and symptoms of dry eye. *Cornea* 2010; 29: 607-612.
- 38 Wang MTM, Tien L, Han A et al. Impact of blinking on ocular surface and tear film parameters. *Ocul Surf* 2018; 16: 424-429.
- 39 Inomata T, Iwagami M, Hiratsuka Y et al. Maximum blink interval is associated with tear film breakup time: A new simple, screening test for dry eye disease. *Sci Rep* 2018; 8: 13443.
- 40 Goto T, Zheng X, Klyce SD et al. A new method for tear film stability analysis using videokeratography. *Am J Ophthalmol* 2003; 135: 607-612.

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Table 1 - Ocular surface demographics characteristics of subjects who participated in this study.

Factor	Value
OSDI score (Median (IQR))	8.30 (4.17 - 12.50)
Osmolarity (Mean \pm SD) [mOsm/l]	303.7 \pm 11.86
Tear meniscus height (Mean \pm SD) [mm]	0.210 \pm 0.045
Corneal fluorescein	
0	33 (82.5 %)
Staining (Oxford scale). n (%)	
1	4 (10.0 %)
2	3 (7.5%)

Table 2 – Descriptive statistics of meibometry, Break-up Time (BUT) and Maximum Blink Interval (MBI) obtained on each inter-week session in n = 40 subjects. SD: Standard Deviation, IQR: Interquartile Range, MU: Meibometer Units, s: seconds.

		Mean	SD	Median	IQR	Minimum	Maximum
Meibometry [MU]	Session 1	452.38	168.53	497.90	331.75 – 559.35	59.80	724.40
	Session 2	428.24	128.95	467.70	321.70 – 513.35	63.80	650.60
BUT [s]	Session 1	9.70	5.69	7.93	4.49 – 11.60	2.83	29.17
	Session 2	11.05	9.29	8.03	5.95 – 10.91	2.37	47.43
MBI [s]	Session 1	12.77	8.19	10.88	7.68 – 15.25	2.07	42.03
	Session 2	15.07	11.32	10.07	8.68 – 15.77	3.55	49.35

Table 3 – Analysis of differences, mean difference and 95% CI between meibometry, Break-up Time (BUT) and Maximum Blink Interval (MBI) results on each inter-week session in n = 40 subjects. SD: Standard Deviation, CI: Confidence Interval, s: seconds.

	Mean difference ± SD	p	95% CI	
			Minimum	Maximum
Meibometry [MU]				
Session 1 – Session 2	24.14 ± 125.07	0.340	245.14	-221.00
BUT [s]				
Session 1 – Session 2	-1.98 ± 9.57	0.326	18.75	-20.74
MBI [s]				
Session 1 – Session 2	-2.30 ± 13.06	0.248	25.59	-27.89

Table 4 - Analysis of differences, mean difference and 95% CI between each inter-week session of Break-up Time (BUT) in n = 33 subjects, considering only BUT values ≤ 15 seconds; and Maximum Blink Interval (MBI) in n = 28, considering only MBI values ≤ 15 seconds. SD: Standard Deviation, CI: Confidence Interval, s: seconds.

		Mean \pm SD	Mean difference \pm SD	p	95% CI	
					Minimum	Maximum
BUT < 15 s [s]	Session 1	7.34 \pm 3.39				
	Session 2	8.10 \pm 3.75	-0.75 \pm 5.33	0.586	10.44	-11.19
MBI < 15 s [s]	Session 1	9.62 \pm 3.48				
	Session 2	9.58 \pm 2.81	0.04 \pm 4.87	0.733	9.55	-9.51

Figure 1 – Bland-Altman plot (Mean vs. Differences) of meibometry between the values obtained in the two inter-week sessions in $n = 40$ subjects. The solid horizontal line indicates the Mean Difference. The two dashed horizontal lines indicate the upper and lower 95% limits of agreement (mean difference $\pm 1.96 \times \text{SD}$). SD: Standard Deviation, MU: Meibometer Units.

Figure 2 – Bland-Altman plot (Mean vs. Differences) of Break-up Time (BUT) between the values obtained in the two inter-week sessions. The solid horizontal line indicates the Mean Difference. The two dashed horizontal lines indicate the upper and lower 95% limits of agreement (mean difference $\pm 1.96 \times \text{SD}$). A) Plot considering all the studied sample ($n = 40$ subjects); the vertical discontinuous line indicates the limit of 15 seconds. B) Plot considering only BUT values ≤ 15 seconds ($n = 33$). SD: Standard Deviation, s: seconds.

Figure 3 – Bland-Altman plot (Mean vs. Differences) of Maximum Blink Interval (MBI) between the values obtained in the two inter-week sessions. The solid horizontal line indicates the Mean Difference. The two dashed horizontal lines indicate the upper and lower 95% limits of agreement (mean difference $\pm 1.96 \times \text{SD}$). A) Plot considering all the studied sample ($n = 40$ subjects); the vertical discontinuous line indicates the limit of 15 seconds. B) Plot considering only MBI values ≤ 15 seconds ($n = 28$). SD: Standard Deviation, s: seconds.