Received: 13 June 2022 | Accepted: 14 November 2022

DOI: 10.1111/opo.13074

OPO THE COLLEGE OF OPTOMETRISTS

ORIGINAL ARTICLE

Repeatability and reproducibility of Cobra HD fundus camera meibography in young adults with and without symptoms of dry eye

Reut Ifrah[1,2] | **Lluisa Quevedo**[2] | **Liat Gantz**[1]

[1]Department of Optometry and Vision Science, Hadassah Academic College, Jerusalem, Israel

[2]Faculty of Optics and Optometry, Universitat Politècnica de Catalunya Terrassa, Barcelona, Spain

Correspondence
Reut Ifrah, Department of Optometry and Vision Science, Hadassah Academic College, Jerusalem, Israel.
Email: reutif@hac.ac.il

Abstract

Purpose: The inter-session repeatability (ISR), inter-examiner reproducibility (IER) and within-subject variability (WSV) of the Cobra HD fundus camera meibographer were examined in participants with and without dry eye symptoms.

Methods: Symptoms were determined based on Ocular Surface Disease Index scores (≥13 being considered symptomatic), and subgroups were compared using the Mann–Whitney *U*-test. Images of meibomian glands (MGs) from the upper and lower right eyelids were captured by two examiners on the same day (S1) to determine IER. One examiner repeated the measurements on a second day (S2) to obtain the ISR. ISR, IER and WSV were calculated using Friedman, correlation tests and Bland and Altman analyses with mean differences (md) and 95% confidence intervals (CIs), within-subject standard deviations (Sw) and intra-class correlation coefficients (ICC).

Results: The ISR experiment included 72 participants (mean age: 23 ± 5 years, range: 19–43, 36 symptomatic). Mean MG loss of the upper (S1: 13.5 ± 9.5%, S2: 12.8 ± 8.5%) and lower eyelids (S1: 7.5 ± 6.9%, S2: 7.3 ± 6.3%) was not significantly different between sessions for all participants, symptomatic and asymptomatic subgroups for both eyelids. The ISR Sw for the upper and lower eyelids was 1.3% and 1.0%; md was 0.7 ± 3.5% (CI:−6.25% to 7.62%) and 0.1 ± 2.1% (CI: −3.94% to 4.17%), respectively. The IER experiment included 74 participants (mean age: 23 ± 5 years, range: 19–43, 37 symptomatic). Mean MG loss of the upper (Examiner 1: 12.7 ± 8.2%, Examiner 2: 13.1 ± 8.0%) and lower eyelids (Examiner 1: 7.0 ± 6.2%, Examiner 2: 7.4 ± 6.2%) was not significantly different between examiners for all participants, symptomatic and asymptomatic subgroups for both eyelids. The IER ICC values were >0.86 for all conditions, Sw was 1.3% and 1.2%, with a md of −0.4 ± 3.2% (CI: −6.65% to 5.90%) and −0.4 ± 2.9% (CI: −6.15% to 5.31%), respectively. The WSV Sw values were <1.4%, and ICC values were >0.89 for both eyelids, examiners and experimental sessions.

Conclusions: The Cobra HD fundus camera demonstrates good repeatability, reproducibility and low WSV, and is a reliable clinical instrument for meibography.

KEYWORDS
Cobra HD fundus camera, meibography, meibomian gland dysfunction, meibomian gland loss, repeatability of meibography, reproducibility of meibography

INTRODUCTION

Meibomian glands (MGs) are large sebaceous glands located in the upper and lower tarsal plates of the eyelids.[1] MGs secrete meibum that forms the outer lipid layer of the tear film and reduces tear evaporation.[2,3] Meibomian gland dysfunction (MGD) is a chronic, diffuse disorder of the MGs, commonly characterised by terminal duct obstruction, as well as qualitative or quantitative changes in the MG secretion.[4] MGD may alter the tear film and cause eye irritation, clinically apparent inflammation and ocular surface disease, such as evaporative dry eye.[4]

Meibomian gland dysfunction diagnosis includes an assessment of symptoms, gland function and morphology. Function is assessed by the meibum quality and expressibility while morphology includes an evaluation of the MGs and eyelid margin abnormalities.[5] Gland morphology can be evaluated using meibography, which is integrated into several types of technologies, including confocal microscopy, optical coherence tomography (OCT) and noncontact infrared photography. The confocal technique requires contact with the eye, potentially resulting in patient discomfort.[6] The OCT does not include the entire MG area within a single image,[7] and is a costly instrument.[8] Noncontact infrared meibography is available as an attachment to the routinely used corneal topographer and fundus photographic equipment. Therefore, this is a more practical option for the screening and follow-up of MGD patients.[6,9]

Infrared meibographers allow observation[10] and in vivo evaluation of the MG morphology,[11] which is useful for both documentation and follow-up.[3]

Several noncontact infrared meibographers are currently available. The OCULUS Keratograph 5 M (OCULUS, Inc., us.oculus.de)[11] employs ImageJ software (imagej.nih.gov) for general image analysis to provide the area of a zone marked by the user.[12] The examiner marks the zones of the everted eyelid and the MGs, and the software provides the area of each zone. The examiner subsequently calculates MG loss by subtraction.[12] However, the two manual steps in this procedure can introduce substantial examiner bias.[11] Despite this, the OCULUS Keratograph 5 M has been reported to have good inter-examiner reproducibility (IER; mean difference between examiners of 0.08 ± 0.55 and 0.13 ± 0.50 grade units in two separate sessions, respectively) with low within-subject variability (WSV; 95% limits of agreement for two different examiners of −1.02 to +1.10 and −1.27 to +1.09 grade units, respectively).[13]

Alternatively, the Cobra HD (csoitalia.it) nonmydriatic digital fundus camera[11] uses Phoenix *semi-automated* software, which is specially designed for MG analysis[14] and employs a 'vectorised' tool with Beziers curves that adapt rapidly to the eyelid shape.[12] Thus, the Phoenix semi-automated software is both quicker and better than the rough segmentation obtained with ImageJ free-hand selection.[12] The Phoenix software requires that the examiner mark the area of the eyelids and the location where the glands are observed, while the software subsequently calculates the percentage of MG loss.[15] Nevertheless, examiners may vary in their identification of the borders of the tarsus and MGs, leading to inter-observer variability.[6,10]

The Cobra HD meibographer has been used previously by at least four research groups in the evaluation of MG morphology.[11,15–17] In most cases, the standard deviations were quite high with respect to the mean measurement.[15–17] Pult[15] examined the relationship between age, sex and dry eye, and MG loss was quantified using the Cobra fundus camera with the Phoenix software digital grading tool in 112 participants. In the Pult study, the mean MG loss for nondry eye vs. dry eye participants resulted in very high standard deviations; 30 ± 17% and 45 ± 18%, respectively. Similarly, another study of dry eye patients also resulted in a high standard deviation,[16] as was the case for patients with Sjorgen syndrome.[17] By contrast, an additional investigation of patients with dry eyes observed low standard deviations (mean standard deviation for both eyelids = 1.57%).[11] If the high standard deviations previously found reflect instrument variability, then this can impact the results and conclusions.[18] Despite the widespread use of the Cobra HD in research studies and the high standard deviations, the inter-session repeatability (ISR) and inter-examiner reproducibility (IER) of this device have not been examined. These outcomes are important when determining the utility of instruments used in comparative and observational studies, and clinical measurements used to diagnose diseases such as MGD.[19] Therefore, this study investigated the ISR, IER and WSV of the Cobra HD fundus camera meibographer using the Phoenix software in participants who were considered either symptomatic or asymptomatic for dry eye based on their Ocular Surface Disease Index (OSDI) questionnaire scores.

Key Points

- The mean difference between the two examiners and measurements taken at two different time points using the Cobra HD fundus camera meibographer was small.
- Meibomian gland loss in the upper eyelid was positively correlated with that seen in the lower eyelid, and while significantly more loss was found in the upper eyelids, there was no significant difference between the symptomatic and asymptomatic subgroups.
- The Cobra HD fundus camera meibographer demonstrated good repeatability, reproducibility and low within-subject variability, making it a reliable clinical instrument.

METHODS

Participants

Participants in this prospective study were recruited through advertisements posted to the student body at Hadassah Academic College (HAC). Those suffering from systemic disease, ocular infection, ocular inflammation or allergies were excluded, as were individuals taking medication that might affect ocular surface symptoms and clinical characteristics (including MG). Pregnant or lactating women were excluded. Participants up to 45 years of age were included to avoid age-related confounding effects since age is a significant risk factor for MGD.[20–22] Contact lens wearers were also excluded as an association between MGD and contact lens wear has been demonstrated.[23]

All examinations took place in the contact lens clinic at the Department of Optometry, HAC, in a designated examination room. The methods were explained verbally and participants signed a statement of informed consent prior to their participation. The study conformed to the tenets of the Declaration of Helsinki and was approved by the HAC Internal Review Board.

Seventy-four participants were measured on the same day by two examiners (Examiner 1, Examiner 2) to determine the IER. Of these, 66 were re-examined by Examiner 1 on a second date to calculate the ISR. An additional six participants were examined by Examiner 1 on two different dates. Their data were included with the 66 participants described above, yielding a total of 72 participants in the ISR experiment (Figure 1).

Experimental procedures

Exclusion criteria were verified based on the medical and ocular history. The OSDI questionnaire was administered to the participants, as it is a widely used questionnaire for dry eye clinical trials.[24] Participants with OSDI scores ≥13 or <13 were classified as symptomatic and asymptomatic, respectively.[24]

The morphology of the upper and lower eyelids can differ,[25] and some studies have reported variations in MG loss from the upper and lower eyelids in patients with MGD.[3,11,26–29] Thus, this investigation compared data from the upper and lower eyelids separately. MGs of the right eyes of the participants were imaged using the Cobra HD fundus camera meibographer, which includes a charge-coupled device high-resolution sensor (2448 × 2051 pixels [5 MPixel]) and an infrared light emitting diode at 850 nm.[11] The upper and lower eyelids were everted while the participant remained stable. After the MGs were seen in focus, the examiner ensured that the lacrimal points were visible and captured three consecutive images per eyelid such that the images were of equivalent magnification. Thus, a total of six images were measured for each participant. Examiners selected and marked both the total area of the everted eyelid and the area containing MGs using the 'vectorised' tool with Beziers curves that conformed to the shape of the eyelid (Figure 2). Then, the Phoenix software improved the contrast[10] and automatically measured the percentage and grade of MG loss according to the meiboscale grading system[12] based on Pult and Nichols.[30]

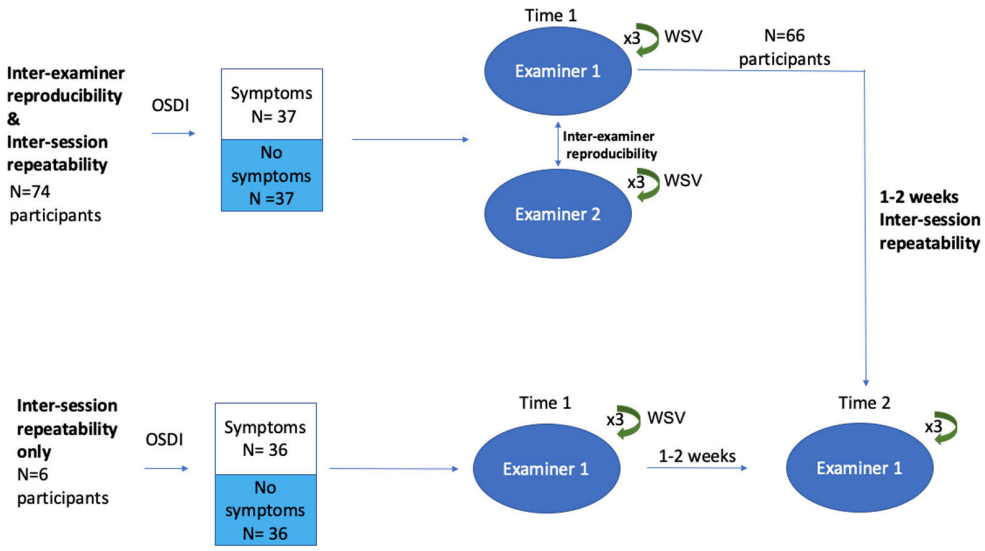

FIGURE 1 Research process. Flow chart of the experimental procedures as described in the text. 74 participants were measured on the same date by two different examiners (Examiner 1, Examiner 2) to determine the inter-examiner reproducibility. Of these 74 participants, 66 were re-examined by Examiner 1 on a second date to calculate the inter-session repeatability (ISR). An additional six participants were examined only by Examiner 1 on two different dates, and their data were included with the 66 participants described above, yielding a total of 72 participants in the ISR experiment. Green arrows represent three consecutive measurements from the same participant that were used to determine within-subject variability (WSV). The Ocular Surface Disease Index (OSDI) questionnaire scores classified participants into symptomatic (≥13) and asymptomatic (no symptoms, scores < 13).

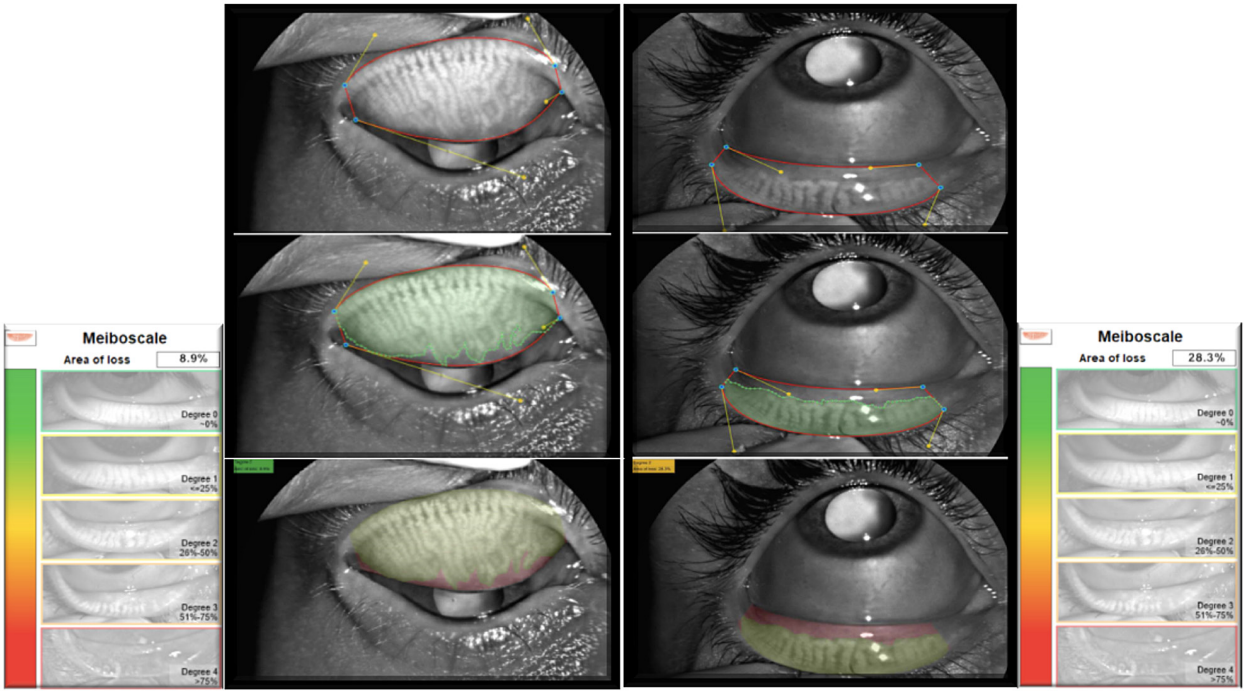

FIGURE 2 Analysis of meibomian gland (MG) loss area by Phoenix software of the upper (left) and lower eyelid (right). Top images: Total MG area measurement. Middle images: Region of interest containing MGs. Bottom images: Calculation of meibomian gland area loss (degree: 0–4, scale: 0%–100%).

This meiboscale characterises the percentage of MG loss (0%–100%) such that 0, 1, 2, 3 and 4 represent no loss, <25% loss, 26%–50% loss, 51%–75% loss and >75% loss, respectively.[12]

Inter-session repeatability

As shown in Figure 1, participants in this substudy ($N = 72$) were retested 1–2 weeks after their initial experimental session at approximately the same time of day in the same designated examination room by Examiner 1.

Inter-examiner reproducibility

As shown in Figure 1, participants in this substudy ($N = 74$) were measured by two examiners during the same experimental session. Participants were measured in a counterbalanced design. Thus, if Examiner 1 was the first to measure the first participant, Examiner 2 was the first to measure the second participant and so on. The examiners were masked as to each other's findings.

Within-subject variability

The WSV was determined from three consecutive measurements of the same eyelid captured by each examiner.

Statistical analysis

Demographic data were evaluated using descriptive statistics. The mean and standard deviation of the percentage of MG loss from the participants' right eyes were calculated. The normality of the outcome measures was assessed using the Kolmogorov–Smirnov test. Data were analysed for the entire sample, and for symptomatic and asymptomatic subgroups separately. Differences between the subgroups were examined for significance using a Mann–Whitney *U*-test that was applied due to a data set that was not normally distributed.

Differences between the upper and lower eyelids were examined using correlation analysis and the Friedman test due to a data set that was not normally distributed.

Differences were considered statistically significant when $p < 0.05$. Statistical analysis was performed with Microsoft Office Excel (microsoft.com) and IBM SPSS Statistics (version.27, ibm.com).

Inter-session repeatability and inter-examiner reproducibility

The ISR and IER were compared using the Friedman test due to non-normal distribution of the outcome measures from the lower eyelids. Both repeatability and reproducibility were determined by calculating the square root of the mean square within groups (Sw), the repeatability limit that is determined by multiplying the Sw by $1.96\sqrt{2}$ (2.77),

which provides an estimate of the limits within which 95% of measurements should lie.[31,32] The intra-class correlation coefficient (ICC) and the 95% confidence intervals (CI) for the ICC were used to determine the IER.[3,33] The ICC ranges from 0–1, with values closer to 1 representing better consistency of measurements.[34]

Pearson's correlation was applied to normally distributed data, and Spearman's correlation was applied to non-normally distributed data. The Bland and Altman analysis[35] was applied to data sets that were significantly correlated. In this analysis, the differences between the measurements of the two sessions or two examiners were plotted against the mean measurement for each subject. It is expected that 95% of differences fall within two standard deviations or less.[35] The mean difference represents the bias, which should be close to zero.[36] The limits of agreement represent the coefficient of repeatability[37] for all comparisons.

Clinically, measurements within 12.5% of one another are considered to be identical, as this is half of the minimum step size of 25% discriminating between the grades of the meiboscale.[30]

Within-subject variability

The WSV was determined by calculating the standard deviation of three consecutive measurements, the Sw and 2.77 Sw of the three consecutive measurements. In addition, the ICC values and the 95% CIs for the ICC were reported.[33]

Sample size

Sample size was calculated per the formulae recommended by McAlinden et al.[31] for three repeated measurements (as in this study). Sample sizes of 43 and 24 participants were required for confidence in the estimate of 15% and 20%, respectively. Thus, with 72 participants, the experiment was sufficiently powered. For the correlation and Bland and Altman analyses, based on Cesana and Antonelli[38] for a power of 0.95, it was necessary to measure 12 subjects for correlation coefficients of 0.85. As our correlation coefficients were all >0.86 and there were 72 pairs of measurements, this study was sufficiently powered.

RESULTS

Inter-session repeatability

Seventytwo participants (mean age: 23.0 ± 4.5 years, range: 19–43, 57 female), 36 symptomatic and 36 asymptomatic were recruited for the ISR experiment. The demographic data of these participants are shown in Table 1.

The mean MG loss (%) from both sessions and both eyelids for all participants and the symptomatic and asymptomatic subgroups are tabulated in Table 2. The MG loss range was 0.6%–49.3% and 0.3%–33.7%, in the upper and lower eyelids, respectively.

The MG loss of the upper eyelids was normally distributed, whereas the MG loss in the lower eyelids as recorded by one examiner was not normally distributed. Therefore, Pearson correlation analysis was used to determine the correlation in the upper eyelids, while Spearman correlation analysis was used in the lower eyelids. The MG loss of the upper and lower eyelids was positively correlated ($p < 0.001$ for all conditions, Table 3) and not significantly different (Friedman test; Table 3, upper eyelids, $p = 0.11, 0.30, 0.32$, lower eyelids, $p = 0.81, 0.74, 1.00$, for all participants, symptomatic and asymptomatic subgroups, respectively) between the first and second sessions.

Meibomian gland loss did not vary significantly between the asymptomatic and symptomatic subgroups (Mann–Whitney *U*-test), for Examiner 1 (upper eyelids, $p = 0.23$, lower eyelids, $p = 0.21$) or Examiner 2 (upper eyelids, $p = 0.22$, lower eyelids, $p = 0.20$). The mean difference that represents the bias[36] and limits of agreements that represent the coefficient of repeatability[37] for all comparisons are tabulated in Table 3 together with Sw and 2.77 Sw. The mean differences (md) between the sessions were 1.20% and below for the entire cohort, asymptomatic and symptomatic subgroups for both upper and lower eyelids. 90.28% and 93.06% of the observations fell within the limits of agreement, in the upper and lower eyelids, respectively.

The Bland and Altman analysis is shown in Figure 3. Only one participant had a difference between the measurements >12.5% in the upper eyelids (99% of the participants had differences in the measurements that were lower than 12.5%), and none of the participants had differences >12.5% in the lower eyelids.

Inter-examiner reproducibility

Seventy-four participants (mean age: 23.4 ± 4.9 years, range: 19–43, 56 female), 37 symptomatic and 37 asymptomatic (Table 4) were included in the IER experiment.

TABLE 1 Demographic data of participants in the inter-session repeatability study

	All participants	Symptomatic	Asymptomatic
N	72	36 (50.0%)	36 (50.0%)
Female	57 (79.2%)	30 (83.3%)	27 (75.0%)
Mean age ± SD (years)	23.0 ± 4.5	22.0 ± 2.4	23.9 ± 5.8
Age range (years)	19–43	19–30	19–43

TABLE 2 Mean meibomian gland (MG) loss (%) in the first and second experimental sessions

	Session 1		Session 2	
Mean MG loss (%)	**Upper eyelid**	**Lower eyelid**	**Upper eyelid**	**Lower eyelid**
All participants ($N = 72$)				
Mean ± SD	13.5 ± 9.5	7.5 ± 6.9	12.8 ± 8.5	7.3 ± 6.3
Range	0.6–49.3	0.3–33.7	0.7–45.8	0.3–29.6
Symptomatic ($N = 36$)				
Mean ± SD	14.5 ± 10.4	8.2 ± 7.4	14.3 ± 9.7	8.0 ± 6.6
Range	1.8–49.3	0.3–31.1	1.8–45.8	0.3–28.6
Asymptomatic ($N = 36$)				
Mean ± SD	12.5 ± 8.6	6.7 ± 6.4	11.3 ± 6.9	6.7 ± 5.9
Range	0.6–39.9	0.6–33.7	0.7–24.9	0.7–29.6

Note: Mean MG loss (%) in the first and second experimental sessions The table displays the means and standard deviations of the mean MG loss (%) measurements of session 1 (left section) and session 2 (right section), as well as the range of measurements for the upper (first and third columns) and lower eyelids (second and fourth columns), for all participants (upper rows), symptomatic participants (middle rows), and asymptomatic participants (bottom rows).

TABLE 3 Statistical outcome measures of inter-session repeatability

Upper eyelid	***R***	***p***	**Mean difference (%) [Range]**	***p***	**Upper 95% CI**	**lower 95% CI**	**Sw**	**2.77 Sw**
All participants ($N = 72$)	0.93	<0.001	0.68 ± 3.54 [−9.93 to 14.97]	0.11	7.62	−6.25	1.26	3.50
Symptomatic ($N = 36$)	0.95	<0.001	0.18 ± 3.35 [−9.93 to 10.67]	0.30	6.75	−6.40	1.18	3.26
Asymptomatic ($N = 36$)	0.91	<0.001	1.19 ± 3.69 [−5.23 to 14.97]	0.32	8.43	−6.05	1.34	3.72
Lower eyelid	ρ	***p***	**Mean difference (%) [Range]**	***p***	**Upper 95% CI**	**lower 95% CI**	**Sw**	**2.77 Sw**
All participants ($N = 72$)	0.89	<0.001	0.12 ± 2.07 [−5.17 to 7.57]	0.81	4.17	−3.94	1.04	2.88
Symptomatic ($N = 36$)	0.86	<0.001	0.27 ± 2.12 [−3.33 to 7.57]	0.74	4.42	−3.88	1.05	2.91
Asymptomatic ($N = 36$)	0.91	<0.001	−0.04 ± 2.04 [−5.17 to 4.13]	1.00	3.97	−4.04	1.03	2.86

Note: The correlation coefficient (Pearson's *R* for upper eyelids and Spearman's ρ for lower eyelids, first column), *p*-value of the correlation analysis (second column), mean difference between the first and second session measurements (third column), *p*-value of the Friedman test comparing between the measurements of the first and second sessions (fourth column), upper (fifth column) and lower (sixth column) limits of agreement, the square root of the mean square within groups (Sw, seventh column) and the repeatability limit within which 95% of the measurements should be ($1.96\sqrt{2}$ [2.77 Sw], eighth column), of the measurements of the first and second sessions.

The mean MG loss (%) measured in both eyelids by both examiners for all participants and the symptomatic and asymptomatic subgroups are tabulated in Table 5. The MG loss range was 0.7%–45.8% and 0.3%–29.6%, in the upper and lower eyelids, respectively.

The MG loss of the upper eyelids was normally distributed, whereas the MG loss of the lower eyelids was not normally distributed. Therefore, Pearson correlation analysis was used to determine the correlation between the examiners for the upper eyelids, while Spearman's correlation analysis examined the relationship between the MG loss of the lower eyelids of the two examiners. The MG loss of the upper and the lower eyelids was positively correlated between the examiners for all conditions ($p < 0.001$) and were not significantly different (Friedman test; Table 6, upper eyelids, $p = 0.32, 0.41, 0.14$, lower eyelids, $p = 0.10, 0.14, 0.40$, for all participants, symptomatic and asymptomatic subgroups, respectively).

The mean difference and limits of agreements for all comparisons are tabulated in Table 6 together with Sw, 2.77 Sw and ICC values. The Bland and Altman analysis can be seen in Figure 4. The md between the examiners were

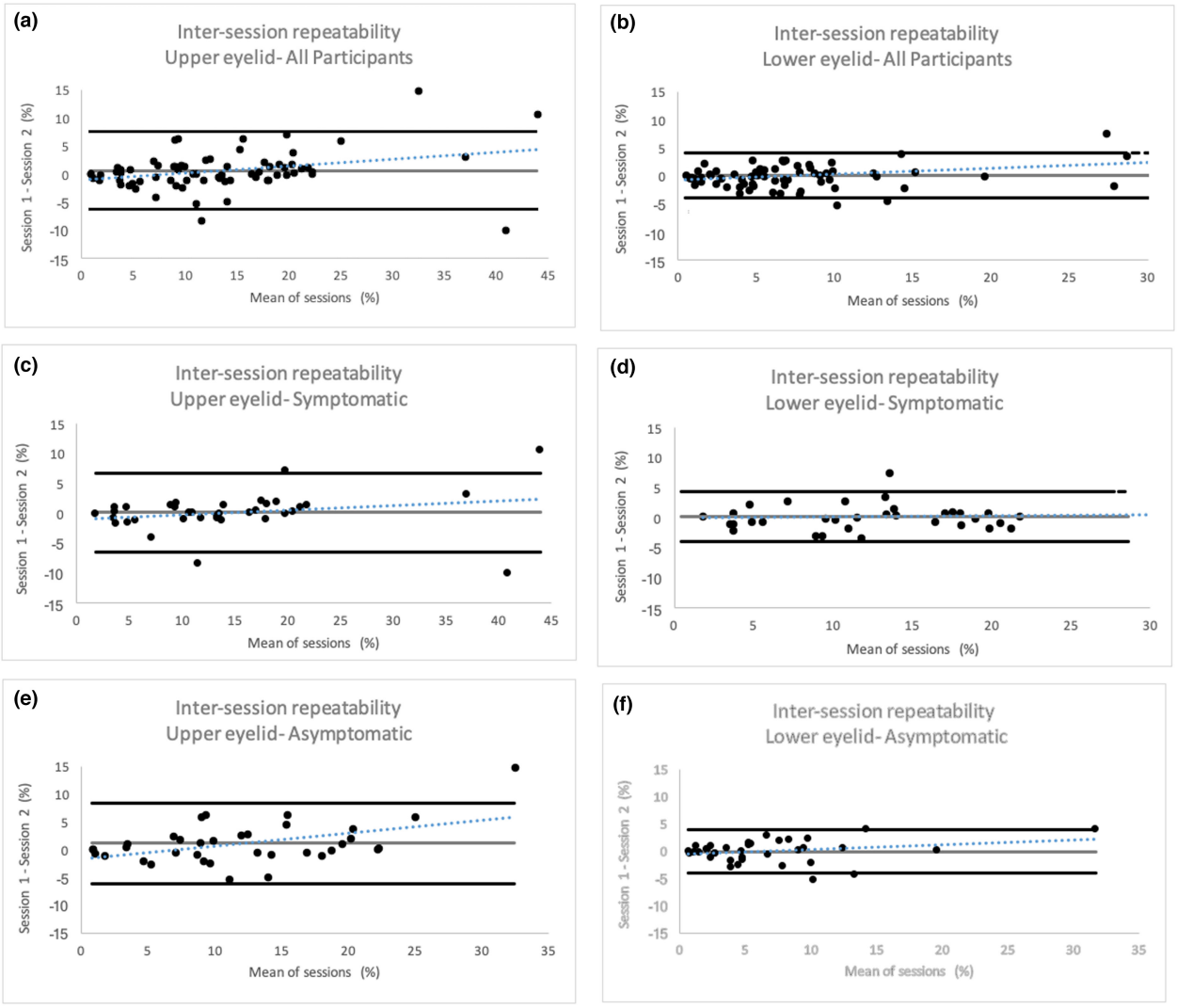

FIGURE 3 Bland and Altman analysis of inter-session repeatability (ISR). Bland and Altman plots of ISR, representing the mean difference in meibomian gland loss in the upper and lower eyelids, for all participants and for symptomatic and asymptomatic subgroups. The grey line represents the mean difference (bias), the black lines show the 95% limits of agreement, and dotted blue lines represent the trendlines fit to the data. Each data point represents one participant. All values presented are in %. (a) Upper eyelid, all participants (b) Lower eyelid, all participants (c) Upper eyelid, symptomatic participants (d) Lower eyelid, symptomatic participants (e) Upper eyelid, asymptomatic participants (f) Lower eyelid, asymptomatic participants.

TABLE 4 Demographic data of participants in the inter-examiner reproducibility study

	All participants	Symptomatic	Asymptomatic
N	74	37 (50%)	37 (50%)
Female	56 (75.7%)	31 (83.8%)	25 (67.6%)
Mean age ± SD	23.4 ± 4.9	22.5 ± 3.5	24.2 ± 5.9
Age range	19–43	19–37	19–43

<0.72% for the entire cohort, asymptomatic and symptomatic subgroups for both upper and lower eyelids. Of the observations, 94.60% fell within the limits of agreement in either eyelid. All participants had differences in measurements between the examiners that were <12.5% for both the upper and lower eyelids.

Within-subject variability

The WSV of the measurements of Examiner 1, for both eyelids and sessions, had Sw values ≤1.27% and ICC values ≥0.93 for all subgroups (Table 7), indicating low WSV. The WSV of the measurements of Examiner 2, for both eyelids and sessions, had Sw values ≤1.34% and ICC values ≥0.89 for all subgroups (Table 7), also indicating low WSV.

Mean MG loss in the upper and lower eyelids as measured by Examiner 1 during the first session were significantly positively correlated for all participants (ρ = 0.57,

TABLE 5 Mean meibomian gland (MG) loss (%) measured by both examiners

	Examiner 1		Examiner 2	
Mean MG loss (%)	**Upper eyelid**	**Lower eyelid**	**Upper eyelid**	**Lower eyelid**
All participants (*N* = 74)				
Mean ± SD	12.7 ± 8.2	7.0 ± 6.2	13.1 ± 8.0	7.4 ± 6.2
Range	0.7–45.8	0.3–29.6	0.5–39.7	0.2–27.7
Symptomatic (*N* = 37)				
Mean ± SD	14.4 ± 9.4	7.9 ± 6.6	14.6 ± 9.0	8.0 ± 6.3
Range	1.8–45.8	0.3–28.6	0.5–39.7	0.3–27.7
Asymptomatic (*N* = 37)				
Mean ± SD	11.1 ± 6.6	6.1 ± 5.8	11.6 ± 6.6	6.8 ± 6.1
Range	0.7–24.9	0.7–29.6	1.0–25.5	0.2–25.7

Note: Mean MG loss (%) measured by both examiners The table displays the means and standard deviations of the mean MG loss (%) measurements of examiner 1 (left section) and examiner 2 (right section), as well as the range of measurements for the upper (first and third columns) and lower eyelids (second and fourth columns), for all participants (upper rows), symptomatic participants (middle rows), and asymptomaticparticipants (bottom rows).

TABLE 6 Statistical outcome measures of inter-examiner reproducibility

Upper eyelid	***R***	***p***	**Mean difference (%) [Range]**	***p***	**Upper 95% CI**	**Lower 95% CI**	**Sw**	**2.77 Sw**	**ICC [95% CI]**
All participants (*N* = 74)	0.92	<0.001	−0.37 ± 3.20 [−12.47 to 8.13]	0.32	5.90	−6.65	1.27	3.51	0.92 [0.88–0.95]
Symptomatic (*N* = 37)	0.94	<0.001	−0.25 ± 3.20 [−8.00 to 8.13]	0.41	6.02	−6.51	1.30	3.59	0.94 [0.89–0.97]
Asymptomatic (*N* = 37)	0.88	<0.001	−0.50 ± 3.24 [−12.47 to 7.13]	0.14	5.86	−6.86	1.24	3.43	0.88 [0.78–0.94]
Lower eyelid	***ρ***	***p***	**Mean difference (%) [Range]**	***p***	**Upper 95% CI**	**lower 95% CI**	**Sw**	**2.77 Sw**	**ICC [95% CI]**
All participants (*N* = 74)	0.82	<0.001	−0.42 ± 2.92 [−10.27 to 7.53]	0.10	5.31	−6.15	1.16	3.23	0.89 [0.83–0.93]
Symptomatic (*N* = 37)	0.77	<0.001	−0.12 ± 2.68 [−6.03 to 7.53]	0.14	5.13	−5.38	1.14	3.16	0.91 [0.84–0.96]
Asymptomatic (*N* = 37)	0.86	<0.001	−0.72 ± 3.16 [−10.27 to 5.90]	0.40	5.47	−6.91	1.19	3.28	0.86 [0.74–0.93]

Note: The correlation coefficient (*R*, first column), *p*-value of the correlation analysis (second column), mean difference between the measurements of the two examiners (third column), *p*-value of the test comparing the measurements of the two examiners (Friedman test for lower eyelids, fourth column), upper (fifth column) and lower (sixth column) limits of agreement, the square root of the mean square within groups (Sw, seventh column), the repeatability limit within which 95% of the measurements should be ($1.96\sqrt{2}$ [2.77 Sw], eighth column) and the intra-class correlation coefficient values (ninth column) between the measurements of the two examiners.

$p < 0.001$), symptomatic participants ($\rho = 0.50$, $p = 0.002$) and asymptomatic participants ($\rho = 0.60$, $p = 0.0003$).

The relationship between the OSDI score (i.e., symptoms of dry eye) and mean MG loss for all subgroups was examined using correlation analysis, as shown in Table 7. Pearson's correlation was applied to the upper eyelids. Spearman correlation was applied for the lower eyelids as the data were not normally distributed. There was no significant association between the OSDI score and mean MG loss for most conditions.

DISCUSSION

This study examined the ISR, IER and WSV of the Cobra HD fundus camera in participants who were classified according to their dry eye symptoms. The mean MG loss in the upper and lower eyelids showed significant positive correlations between the first and second sessions and between the two examiners. In addition, no significant difference was found between the repeated measurements, in both sessions and for both examiners, indicating good ISR and

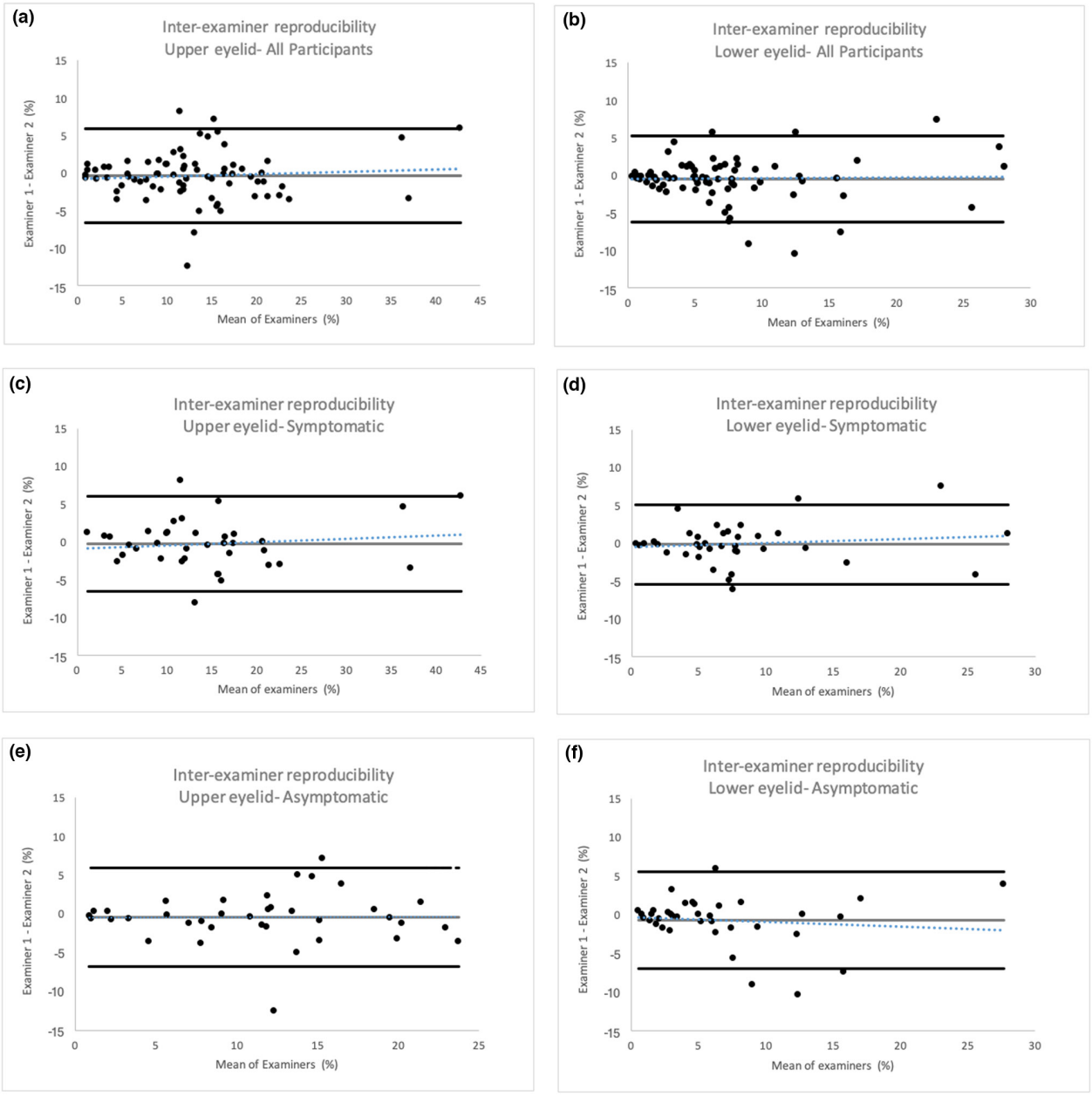

FIGURE 4 Bland and Altman analysis of inter-examiner reproducibility (IER). Bland and Altman plots of IER, representing the mean difference in meibomian gland loss in the upper and lower eyelids, for all participants and for symptomatic and asymptomatic subgroups. The grey line represents the mean difference (bias), the black lines show the 95% limits of agreement, and dotted blue lines represent the trendlines fit to the data. Each data point represents one participant. All values presented are in %. (a) Upper eyelid, all participants (b) Lower eyelid, all participants (c) Upper eyelid, symptomatic participants (d) Lower eyelid, symptomatic participants (e) Upper eyelid, asymptomatic participants (f) Lower eyelid, asymptomatic participants.

IER. The WSV of the measurements for both examiners, both eyelids and sessions had Sw values of ≤1.34% and ICC values ≥0.89 for all subgroups, indicating low WSV.

Clinical significance

For the Cobra HD fundus camera, MG loss was classified using the meiboscale developed by Pult and Nichols,[30] and clinical treatment is based on the meiboscale grading.[39] As noted earlier, the step size between levels in this grading scale is approximately 25%.[30] Therefore, if there are differences between examiners or between sessions that are equivalent to 25% or greater, then this would be a clinically significant result. As such, in the present investigation, we considered differences that were half that step size, i.e., 12.5%, to be clinically insignificant.

The mean MG loss in the ISR and IER experiments for all participants as well as the symptomatic and asymptomatic subgroups was greater in the upper than the lower eyelid.

TABLE 7 Statistical outcome measures of within-subject variability and correlations between Ocular Surface Disease Index questionnaire scores and mean meibomian gland loss

	Upper eyelid					Lower eyelid				
	Sw	2.77 Sw	ICC [95% CI]	*R*	*p*	Sw	2.77 Sw	ICC [95% CI]	*ρ*	*p*
Examiner 1										
Session 1										
All participants (*N* = 72)	1.15	3.18	0.96 [0.94–0.97]	0.14	0.25	1.04	2.89	0.95 [0.92–0.97]	0.24	0.05
Symptomatic (*N* = 36)	1.27	3.51	0.96 [0.93–0.98]	0.05	0.76	1.10	3.04	0.96 [0.93–0.98]	0.18	0.30
Asymptomatic (*N* = 36)	1.01	2.80	0.97 [0.94–0.98]	0.27	0.11	0.98	2.72	0.93 [0.89–0.96]	0.34	0.06
Session 2										
All participants (*N* = 74)	1.17	3.25	0.97 [0.95–0.98]	0.15	0.17	1.09	3.02	0.95 [0.93–0.97]	0.26	0.02
Symptomatic (*N* = 37)	1.19	3.31	0.96 [0.93–0.98]	0.01	0.94	1.13	3.13	0.96 [0.93–0.98]	0.16	0.35
Asymptomatic (*N* = 37)	1.15	3.20	0.97 [0.95–0.99]	0.18	0.25	1.04	2.89	0.94 [0.89–0.97]	0.23	0.17
Examiner 2										
All participants (*N* = 74)	1.29	3.57	0.93 [0.91–0.96]	0.24	0.04	1.22	3.37	0.91 [0.87–0.94]	0.23	0.05
Symptomatic (*N* = 37)	1.23	3.42	0.96 [0.93–0.98]	0.13	0.45	1.28	3.56	0.91 [0.86–0.95]	0.07	0.69
Asymptomatic (*N* = 37)	1.34	3.71	0.89 [0.81–0.93]	0.37	0.02	1.15	3.17	0.90 [0.83–0.94]	0.25	0.13

Note: The square root of the mean square within groups (Sw, first and sixth columns), and the repeatability limit within which 95% of the measurements should lie (1.96√2 [2.77 Sw], second and seventh columns), intra-class correlation coefficient (ICC) values (third and eighth columns) of the measurements, correlation coefficients (Pearson's *R* for upper eyelids and Spearman's *ρ* for the lower eyelids, fourth and ninth columns), and *p*-values (*p*, fifth and tenth columns) of the correlations of the upper (left section) and lower eyelids (right section) for each examiner are tabulated. Blue cells represent significant correlations.

This finding is similar to AlDarrab et al.,[26] who reported MG loss of 0.22 ± 0.54% in the upper versus 0.14 ± 0.35% in the lower eyelids. This finding is also similar to Golebiowski et al.[27] who reported MGD scores of 7.3 ± 6.2 and 2.0 ± 2.8 in the upper and lower eyelids, respectively. However, it differs from the findings of Garduno et al.[11] who compared MG loss quantified using the Cobra HD versus Antares devices (csoitalia.it) in 80 participants with (*N* = 26) and without evaporative dry eye (*N* = 54), based on the TFOS DEWS II classification criteria. They reported higher MG loss in the lower compared with the upper eyelids for all subgroups except the evaporative dry eye subgroup. These discrepant findings may be due to differences in the age of the cohorts in the two studies. The mean age in the present investigation was 23 years, ranging between 19–43 years, whereas the Garduno et al.[11] study cohort had a mean age of 37 years, ranging between 18–78 years. Differences in methodology could also account for the discrepant findings. In the present investigation, lid eversion was achieved manually. Conversely, as described in their discussion, Garduno et al.[11] used a device to evert the eyelids. Thus, the varying techniques may expose different areas of the eyelids leading to discrepancies in the measurements.

Inter-examiner reproducibility of mean MG loss was better for the upper than the lower eyelid, as the correlation and ICC values were higher (Table 6). This is in accordance with Dogan et al.,[6] who reported moderate to good agreement between examiners for the upper eyelid and fair to moderate agreement for the lower eyelid using the Sirius (CSO, csoitalia.it) corneal topographer on 30 outpatient clinic subjects. Differences in reproducibility values of MG loss from the upper versus lower eyelids may be due to the morphological diversity of the two eyelids.[25] Although lower eyelid evaluation appears to be more practical because of the ease of eversion, the excessive area over the tarsus can be erroneously marked due to the laxity of the lower eyelid in the free-hand tool.[6]

Despite the morphological variations of MGs in the upper and lower eyelids, the present study found, similar to Pult et al.,[29] that the MG loss in the upper and lower eyelids of all experimental subgroups showed a significant positive correlation. Furthermore, MG loss did not vary significantly between symptomatic and asymptomatic subgroups, in either eyelid, for both examiners.

In the present study, there was no association between OSDI scores and mean MG loss for most experimental conditions. This finding is consistent with other studies that did not find a correlation between OSDI scores and dry eye signs.[6,40] For example, Machalińska et al.[41] analysed the association between MG characteristics and tear film-related factors and found that OSDI scores did not correlate with functional and morphological MG parameters in contact

lens wearers. Additionally, Dogan et al.[6] found no correlation between OSDI scores and MG loss rate, for both the upper and lower eyelids, while investigating the inter-examiner reliability of meibography evaluation and the impact of eyelid selection for the procedure. Adil et al.[40] investigated the relationship between MG morphology and clinical dry eye tests in patients with MGD and found no correlation between OSDI and any MG morphological parameter. However, it should be noted that the OSDI questionnaire does not differentiate aqueous deficiency from evaporative dry eye disease, which is most commonly caused by MGD.[41] Additionally, Adil et al.[40] suggested that MG loss is a morphological sign that reflects the early stages of MGD, which precedes symptoms.

The findings of this study are comparable with previous studies of MGD using the Phoenix software for image analysis, which is included in the Cobra HD fundus camera, the Antares topographer and the Sirius Scheimpflug camera topographer.[8] Dogan et al.[6] examined the IER between three examiners using the Sirius corneal topographic device. They observed a md of 4% in the upper eyelids and 1% in the lower eyelids with ICC values of 0.87 and 0.85 for the upper and lower eyelids, respectively. Thus, they concluded that the Sirius corneal topographic device provides moderate to good agreement of MG loss of the upper eyelids and fair to moderate agreement of MG loss in the lower eyelids.

Garza-Leon et al.[12] evaluated the agreement between two different software tools, i.e., Phoenix and ImageJ using the Antares meibographer, each analysed by two different examiners. In their investigation, only one set of photographs was collected from each participant and was subsequently analysed offline by two separate examiners. While not their primary study goal, they reported a WSV (ICC) of 0.99. They also reported a mean difference between observers when assessing the same image using the Phoenix program of 0.45% MG loss. In the Garza-Leon et al.[12] study, two examiners assessed one set of images from each participant. By contrast, in the present study, each examiner captured and analysed their own images of the subjects. These methods more closely resemble the clinical setting in which examiners both capture and analyse the images. The mean difference between the MG loss assessed by the two examiners for all participants was $-0.37 \pm 3.20\%$ and $-0.42 \pm 2.29\%$ for the upper and lower eyelids, respectively. This is similar to the mean difference between two examiners assessing the same image (0.45%) reported by Garza-Leon et al.[12] Thus, our findings indicate that the repeatability of the Cobra HD instrument is similar to other meibographers evaluating MG loss using the Phoenix software.

Although the Cobra HD fundus camera meibographer has been previously compared with the Antares instrument and they were found to be interchangeable,[11] the Cobra HD device has not been compared with other commonly used meibographers such as the OCULUS Keratograph. Future studies should evaluate the interchangeability of these instruments.

CONCLUSIONS

In conclusion, the present study examined the IER, ISR and WSV of MG loss quantified using the Cobra HD fundus camera meibographer. Differences between the examiners and sessions were <12.5%, which is half of the minimum step size when discriminating between grades on the meiboscale.[30] Thus, the Cobra HD fundus camera meibographer demonstrates good repeatability and reproducibility, and clinically similar findings should be obtained when used by different examiners on different occasions. Thus, it is suitable for the meibographic assessment and follow-up of disease progression or treatment outcomes.

AUTHOR CONTRIBUTIONS

Reut Ifrah: Conceptualization (equal); data curation (equal); formal analysis (lead); investigation (equal); methodology (equal); project administration (equal); resources (equal); validation (equal); writing – original draft (lead); writing – review and editing (equal). **Luisa Quevedo:** Conceptualization (equal); supervision (equal); visualization (equal); writing – review and editing (equal). **Liat Gantz:** Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); resources (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (supporting); writing – review and editing (equal).

ACKNOWLEDGEMENTS

We would like to thank Prof. Ariela Gordon-Shaag, for her assistance with this article, Mr. Yair Raiz for statistical assistance and Mrs. Gal Hazrati for her help in performing the tests.

CONFLICT OF INTEREST

The authors have no conflicts to disclose.

ORCID

Reut Ifrah https://orcid.org/0000-0001-7924-0610
Liat Gantz https://orcid.org/0000-0002-9604-3528

REFERENCES

1. Harbiyeli II, Bozkurt B, Erdem E, Ozcan HG, Cam B, Sertdemir Y, et al. Associations with meibomian gland loss in soft and rigid contact lens wearers. *Cont Lens Anterior Eye*. 2022;45:101400. https://doi.org/10.1016/j.clae.2020.12.005.
2. Gu T, Zhao L, Liu Z, Zhao S, Nian H, Wei R. Evaluation of tear film and the morphological changes of meibomian glands in young Asian soft contact lens wearers and non-wearers. *BMC Ophthalmol*. 2020;20:84. https://doi.org/10.1186/s12886-020-1328-2
3. Gulmez Sevim D, Gumus K, Unlu M. Reliable, noncontact imaging tool for the evaluation of meibomian gland function: Sirius Meibography. *Eye Contact Lens*. 2020;46:S135–40.
4. Nelson JD, Shimazaki J, del Benítez Castillo JM, Craig JP, Mcculley JP, Den S, et al. The international workshop on meibomian gland

dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci*. 2011;52:1930–7.
5. Lin X, Fu Y, Li L, Chen C, Chen X, Mao Y, et al. A novel quantitative index of meibomian gland dysfunction, the meibomian gland tortuosity. *Transl Vis Sci Technol*. 2020;9:34. https://doi.org/10.1167/tvst.9.9.34
6. Dogan AS, Kosker M, Arslan N, Gurdal C. Interexaminer reliability of meibography: upper or lower eyelid? *Eye Contact Lens*. 2018;44:113–7.
7. Yoo YS, Na KS, Byun YS, Shin JG, Lee BH, Yoon G, et al. Examination of gland dropout detected on infrared meibography by using optical coherence tomography meibography. *Ocul Surf*. 2017;15:130–8.e1.
8. Chopra R, Wagner SK, Keane PA. Optical coherence tomography in the 2020s-outside the eye clinic. *Eye*. 2021;35:236–43.
9. Daniel E, Maguire MG, Pistilli M, Bunya VY, Massaro-Giordano GM, Smith E, et al. Grading and baseline characteristics of meibomian glands in meibography images and their clinical associations in the dry eye assessment and management (DREAM) study. *Ocul Surf*. 2019;17:491–501.
10. Arita R, Suehiro J, Haraguchi T, Shirakawa R, Tokoro H, Amano S. Objective image analysis of the meibomian gland area. *Br J Ophthalmol*. 2014;98:746–55.
11. Garduño F, Salinas A, Contreras K, Rios Y, García N, Quintanilla P, et al. Comparative study of two infrared meibographers in evaporative dry eye versus nondry eye patients. *Eye Contact Lens*. 2021;47:335–40.
12. Garza-Leon M, Gonzalez-Dibildox A, Ramos-Betancourt N, Hernandez-Quintela E. Comparison of meibomian gland loss area measurements between two computer programs and intra-inter-observer agreement. *Int Ophthalmol*. 2020;40:1261–7.
13. Ngo W, Srinivasan S, Schulze M, Jones L. Repeatability of grading meibomian gland dropout using two infrared systems. *Optom Vis Sci*. 2014;91:658–67.
14. García-Marqués JV, Macedo-De-Araújo RJ, Cerviño A, García-Lázaro S, González-Méijome JM. Assessment of meibomian gland drop-out and visibility through a new quantitative method in scleral lens wearers: a one-year follow-up study. *Cont Lens Anterior Eye*. 2022;101571. [Epub ahead of print]. https://doi.org/10.1016/j.clae.2021.101571
15. Pult H. Relationships between meibomian gland loss and age, sex, and dry eye. *Eye Contact Lens*. 2018;44:S318–24.
16. Sánchez-González JM, De-Hita-Cantalejo C, Sánchez-González MC. Crosslinked hyaluronic acid with liposomes and crocin for management symptoms of dry eye disease caused by moderate meibomian glan d dysfunction. *Int J Ophthalmol*. 2020;13:1368–73.
17. Fondi K, Miháltz K, Vécsei-Marlovits PV. Efficacy of topical hydrocortisone in combination with topical Ciclosporin a for the treatment of dry eye disease in patients with Sjögren syndrome. *J Ophthalmol*. 2021;2021:7584370. https://doi.org/10.1155/2021/7584370
18. Barde MP, Barde PJ. What to use to express the variability of data: standard deviation or standard error of mean? *Perspect Clin Res*. 2012;3:113–6.
19. Powell DR, Nichols JJ, Nichols KK. Inter-examiner reliability in meibomian gland dysfunction assessment. *Invest Ophthalmol Vis Sci*. 2012;53:3120–5.
20. Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology*. 2008;115:911–5.
21. Den S, Shimizu K, Ikeda T, Tsubota K, Shimmura S, Shimazaki J. Association between meibomian gland changes and aging, sex, or tear function. *Cornea*. 2006;25:651–5.
22. Mathers WD, Lane JA. Meibomian gland lipids, evaporation, and tear film stability. *Adv Exp Med Biol*. 1998;438:349–60.
23. Ifrah R, Quevedo L, Gantz L. Topical review of the relationship between contact lens wear and meibomian gland dysfunction. *J Optom*. 2022;S1888-4296(22)00017-6. https://doi.org/10.1016/j.optom.2022.03.004
24. Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, et al. TFOS DEWS II diagnostic methodology report. *Ocul Surf*. 2017;15:539–74.
25. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci*. 2011;52:1938–78.
26. AlDarrab A, Al-Faky Y, Mousa A, Alsuhaibani A. Meibography of eyes with sequelae of trachoma. *Cornea*. 2018;37:416–20.
27. Golebiowski B, Chim K, So J, Jalbert I. Lid margins: sensitivity, staining, meibomian gland dysfunction, and symptoms. *Optom Vis Sci*. 2012;89:1443–9.
28. McCann LC, Tomlinson A, Pearce EI, Diaper C. Tear and meibomian gland function in blepharitis and normals. *Eye Contact Lens*. 2009;35:203–8.
29. Pult H, Riede-Pult BH, Nichols JJ. Relation between upper and lower lids' meibomian gland morphology, tear film, and dry eye. *Optom Vis Sci*. 2012;89:E310–5.
30. Pult H, Nichols JJ. A review of meibography. *Optom Vis Sci*. 2012;89:E760–9.
31. McAlinden C, Khadka J, Pesudovs K. Precision (repeatability and reproducibility) studies and sample-size calculation. *J Cataract Refract Surg*. 2015;41:2598–604.
32. Vaz S, Falkmer T, Passmore AE, Parsons R, Andreou P. The case for using the repeatability coefficient when calculating test-retest reliability. *PLoS One*. 2013;8:e73990. https://doi.org/10.1371/journal.pone.0073990
33. Nichols JJ, Berntsen DA, Mitchell GL, Nichols KK. An assessment of grading scales for meibography images. *Cornea*. 2005;24:382–8.
34. Ning R, Gao R, Piñero DP, et al. Repeatability and reproducibility of corneal higher-order aberrations measurements after small incision lenticule extraction using the Scheimpflug-Placido topographer. *Eye Vis*. 2022;9:1. https://doi.org/10.1186/s40662-021-00274-y
35. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1:307–10.
36. Giavarina D. Understanding Bland Altman analysis. *Biochem Med*. 2015;25:141–51.
37. Loughman J. The statistical interpretation of the coefficient of repeatability. *Br J Ophthalmol*. 2010;94:753–6.
38. Cesana BM, Antonelli P. Agreement analysis: further statistical insights. *Ophthalmic Physiol Opt*. 2012;32:436–9; author reply 439–40.
39. Nichols KK, Foulks GN, Bron AJ, Glasgow BJ, Dogru M, Tsubota K, et al. The international workshop on meibomian gland dysfunction: executive summary. *Invest Ophthalmol Vis Sci*. 2011;52:1922–9.
40. Adil MY, Xiao J, Olafsson J, Chen X, Lagali NS, Ræder S, et al. Meibomian gland morphology is a sensitive early indicator of meibomian gland dysfunction. *Am J Ophthalmol*. 2019;200:16–25.
41. Machalińska A, Zakrzewska A, Safranow K, Wiszniewska B, Machaliński B. Risk factors and symptoms of meibomian gland loss in a healthy population. *J Ophthalmol*. 2016;2016:7526120. https://doi.org/10.1155/2016/7526120

How to cite this article: Ifrah R, Quevedo L, Gantz L. Repeatability and reproducibility of Cobra HD fundus camera meibography in young adults with and without symptoms of dry eye. *Ophthalmic Physiol Opt*. 2022;00:1–12. https://doi.org/10.1111/opo.13074