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# Comparison of subjective grading and objective assessment in meibography

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ABSTRACT

*Aim:* To analyse repeatability of subjective grading and objective assessment in non-contact infra-red meibography.
*Methods:* Meibography photographs of 24 subjects (female 14; mean age = 46; range = 19–69 years, upper-lid images = 12, lower-lid images = 12) were classified in two sessions by three experienced observers (OI, OII, OIII). Relative area or portion affected by meibomian glands (MG) loss was classified applying three different grading scales in randomized order: a four-grade scale (4S) (degree 0 = no partial glands; 1 = <25% partial glands; 3 = 25–75% partial glands; 3 = >75% partial glands), a pictorial five-grade scale (5S) (degree 0 = no meibomian gland loss (MGL); 1 = <25% MGL; 3 = 26–50% MGL; 3 = 51–75%; 4 = >75% MGL) and objectively by a 100-grade scale (DA) applying ImageJ software.
*Results:* Observed MG loss ranged from 0% to 69%. Intra-observer agreement of the 5S (OI: $\kappa = 0.80$, $p < 0.001$; OII: $\kappa = 0.40$, $p = 0.009$; OIII $\kappa = 0.81$, $p < 0.001$) was better than of the 4S (OI: $\kappa = 0.79$, $p < 0.001$; OII: $\kappa = 0.15$, $p = 0.342$; OIII $\kappa = 0.50$, $p = 0.0071$). Intra-observer agreement of OI and OIII (±0.88 (95% confidence interval), ±1.305) was better than of OII (±2.21) in 4S and 5S (±0.99, ±2.00 and ±0.91; OI, OII and OIII, respectively) while it was relatively similar in DA (±18, ±17 and ±17). Inter-observer agreement was better in DA (OI–OII: ±13, OI–OII: ±19, OII–OIII: ±26) than in 4S (OI–OII: ±1.76; OI–OIII: ±1.29 and OII–OIII: ±1.31) or 5S (OI–OII: ±1.49; OI–OIII: ±0.91 and OII–OIII: ±1.20).
*Conclusion:* Intra-observer and inter-observer agreement was better in computerized grading followed by the subjective five-grade scale and four-grade scale.


## 1. Introduction

Meibomian gland dysfunction (MGD) is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion [1]. MGD results in stasis of meibum inside the glands, dilatation of the ductal system and loss of glandular tissue [2]. Meibography is a well-known technique for the assessment of meibomian gland morphology, meibomian gland changes and the diagnoses of Meibomian gland dysfunction [3–17].

Tapie [12] was probably first describing meibography using transillumination of the everted eye lid followed by many other researchers [4,8,10,11,17]. Non-contact meibography was introduced by Arita et al. making meibography much more comfortable for the patient [6,14]. In this technique the everted eye lid is not touched by an instrument. The lid is illuminated by infra-red (IR) light from a slit lamp and captured via an IR video camera mounted on the slit lamp microscope (SLM). SLM independent systems are the portable non-contact meibograph (PNCM) – which is a small IR Camera including an IR light source – and the EyeTop® Topographer, Sirius® Scheimpflug Camera and Cobra® Fundus Camera (CSO, Costruzione Strumenti Oftalmici, Florence, Italy; bon Optic VertriebsgmbH, Lübeck) [18] and the Oculus Keratograph® (Oculus, Wetzlar, Germany) all including meibography options using their internal IR cameras [14,16,19,20].

Normal meibomian glands appear as grapelike clusters with acini that are IR hyper-reflective [8,14]. Many different grading scales have been published; all of them are four-grade scales [6,8,21]. Therefore, a verbal and pictorial five-grade scale (Fig. 1) was developed in order to enhance the grading in meibography, making it more sensitive with to smaller increments [14]. Additionally, computerized grading of meibomian gland morphology was reported, measuring the "area of loss of MG" applying ImageJ 1.42q (Wayne Rasband, National Institute of Health, Bethesda, MD).

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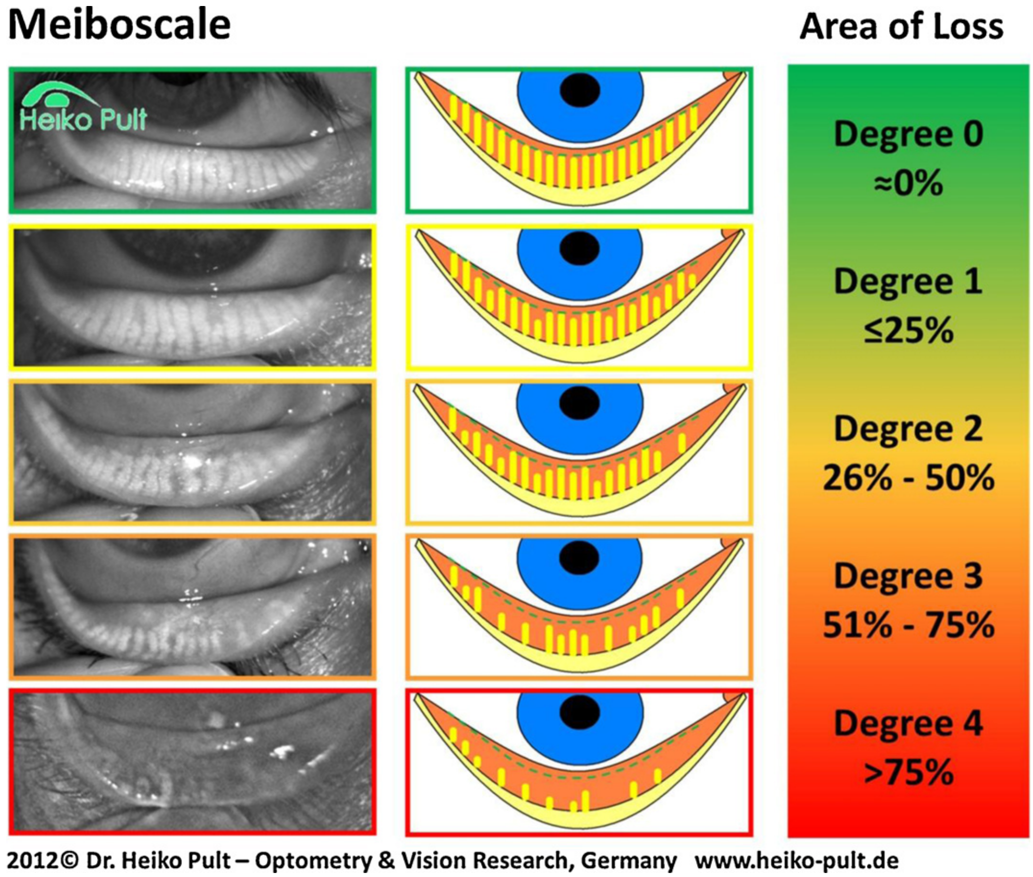


**Fig. 1.** Five-grade meiboscale [14].

This technique is suggested to be useful for the evaluation of dry eye and MGD [14,16,20,22,23].

Meibography appears to be a valuable clinical test in the diagnoses of dry eye and meibomian gland dysfunction [3,14,23,24]. However to obtain meibography in clinical practice an effective grading system seems to be vital [8,14].

The aim of this pilot-study was to evaluate (i) if a five-grade scale improves inter- and intra-observer repeatability of the subjective classification in meibography and (ii) if computerized grading [14,16,23] is superior to subjective grading.

## 2. Methods

Twenty-four subjects (female 14; mean age = 46; range = 19–69 years) were randomly selected from the patient pool of the Horst Riede GmbH, Weinheim, Germany for participation. Subjects were excluded if they had diabetes, recent ocular infections, seasonal allergies, any history of ocular surgery, use of any medication or eye drops known to affect the ocular surface, were a current or prior contact lens wearer, or were pregnant by self-report. All procedures were conducted in accordance with the Declaration of Helsinki (1983). All subjects provided written informed consent before participating in the study. Subject number was calculated by power calculation (aimed power: >80%, difference: 0.50 between subjective grades) and of desired confidence interval (<0.5) [25] based on published figures [8].

### 2.1. Meibography

Non-contact infra-red meibography was performed on one randomly selected eye of each subject using the portable non-contact meibograph (PNCM) [16,23]. The PNCM was connected to a computer via a Video-to-FireWire Converter DFG/1394-1e (The Image Source Europe GmbH, Bremen, Germany) and photographs were captured by IC Capture 2.0 and IC Imaging Control 3.1 (The Image Source Europe GmbH) and displayed on a 22 in. TFT screen. Twelve upper and twelve lower lid meibography images were randomly selected for classification. Apart from randomization, no further criteria were applied for image selection.

### 2.2. Grading

Meibography images were classified by three experienced observers (OI, OII, OIII). Each image was classified applying two different grading scales and digital analyses (DA) applying ImageJ 1.42q (Wayne Rasband, National Institute of Health, USA; http://rsbweb.nih.gov/ij/). Grading of the meibography images was repeated on the following day. Order of evaluation and scales was randomized; observers were masked against each other and sessions.

#### 2.2.1. Subjective grading

For the subjective classification of meibography images a four-grade scale [8] (degree 0 = no partial glands; 1 = <25% partial glands; 3 = 25–75% partial glands; 3 = >75% partial glands) and a new five-grade scale [14] (Fig. 1) were applied. The criteria of “partial glands” of the four-grade scale are defined as following [8]. Complete meibomian glands are those that traverse the lid linearly roughly 3–4 mm; those that do not traverse the lid fully or are found in small, irregular clumps are termed “partial” meibomian glands [8]. The criterium “loss of meibomian glands (MGL)” was defined as the proportion of the area of MG loss (Fig. 2B) in its relation to the total

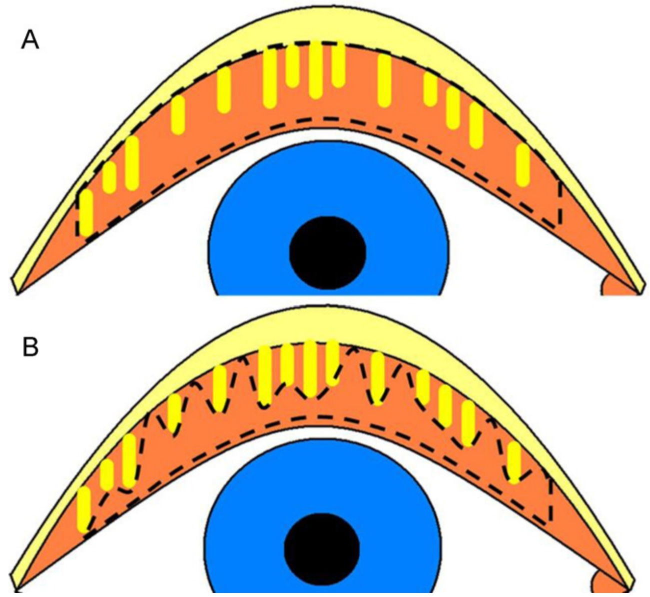


**Fig. 2.** Definition of total area of meibomian glands (A) and area of loss (B) on which subjective and computerized grading is based on.

area of glands if the subject would have had normal meibomian glands (Fig. 2A) [6,16,22,23,26].

#### 2.2.2. Objective assessment

Applying the computerized grading, the area of MG loss was measured with the ImageJ software and its relation to the total-area was noted as fraction (score: 0–100 MGL) [16,23]. This factor is named MG loss (Fig. 3) [16,20,23].

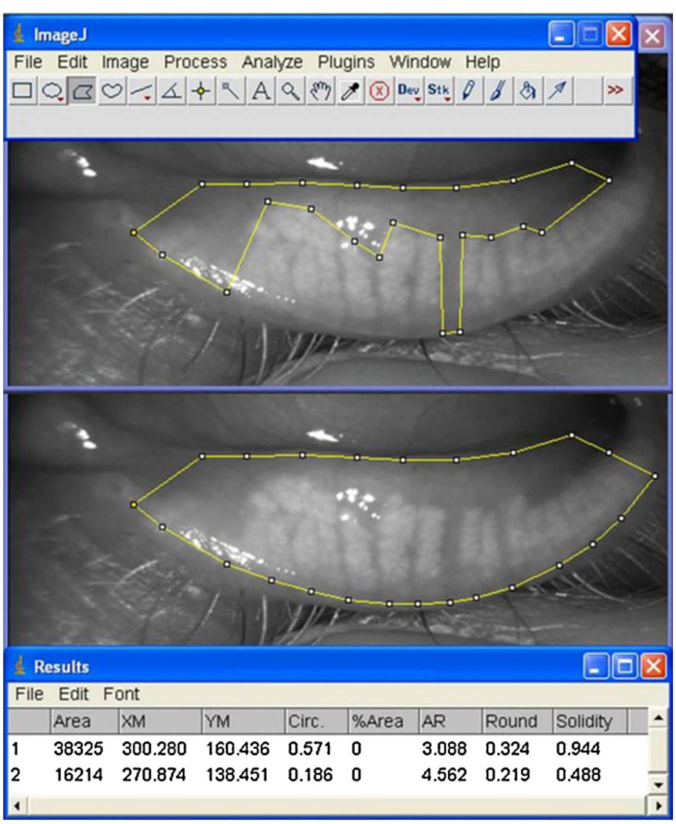


**Fig. 3.** Computerized grading, applying ImageJ software [14].

### 2.3. Statistical analyses

Normal distribution of data were analysed by Shapiro–Wilk test. Differences between observers and session were analysed by repeated measurements ANOVA (Friedman test in ordinal scale or non-parametric data) and Bland–Altman plots and relations by Pearson correlation (Spearman rank in ordinal scale or non-parametric data). 95% confidence intervals (CI) were calculated from the distributions of differences between observer and observations. For the subjective grading scales Cohen's weighted $\kappa$-statistic was applied. Kappa-statistic was not performed for the computerized grading. If one of the examiners did not assigned a grade at least once in each of the categories kappa could not be calculated. Moving one of the observations into that category or group categories together would have induced bias.

The data were analysed using BiAS 9.05 (Espilon Verlag, Frankfurt, Germany) and SPSS 20.0 (SPSS Inc., Chicago, USA).

## 3. Results

Observed MG loss scores ranged from 0 to 3 applying the four grade-scale, 0 to 4 in the five-grade scale and 0 to 69 in computerized grading. No significant differences were found between sessions ($p > 0.168$) and observers (Table 1A).

### 3.1. Subjective grading

#### 3.1.1. Weighted $\kappa$-statistic

Kappa was better of the five-grade scale than of the four-grade scale (Table 2).

#### 3.1.2. Intra-observer agreement

95% confidence interval (CI) of OI and OIII was better than of OII in the subjective grading scales (Table 1B). Agreement of the five-grade scale was similar to the CI of the four-grade scales. CI in OII and OIII was better for the five-grade scale compared to the four-grade scale. CI Sessions of OI and OIII correlated well for all grading systems ($r > 0.70$; $p < 0.001$) but not in OII (Table 1C). Sessions of OII were not correlated when applying the four-grade scale (Spearman rank, $r = 0.16$; $p = 0.214$) but were significantly correlated with the five-grade scale ($r = 0.58$; $p = 0.001$).

#### 3.1.3. Inter-observer agreement

CI was slightly better for the five-grade scale compared to the four-grade scale but best for the computerized grading (Figs. 4–6; Table 1B). Correlations between observers were better with the five-grade scale and computerized grading than of the four-grade scale (Table 1C).

### 3.2. Objective assessment

#### 3.2.1. Intra-observer agreement

Limit of agreement (CI) was better with the computerized grading than with subjective grading scales (Tables 1B and 3) and sessions of all observers were significantly correlated with the computerized grading ($r > 0.81$; $p < 0.001$).

#### 3.2.2. Inter-observer agreement

CI was better with the digital analyses than with the subjective grading scales (Figs. 4–6; Tables 1B and 3). Correlations between observers were similar to the five-grade scale but better than of the four-grade scale (Table 1C).

**Table 1**
(A) Mean grade and standard deviation (SD) classified by each grading system (†Friedman test, ††ANOVA repeated measurements). (B) 95% confidence interval (CI). (C) Correlation between observers and sessions of each grading system (†Spearman rank; ††Pearson correlation) (observer = O; session = S).

| (A) Mean grade | Four-grade scale | | | Five-grade scale | | | Computerized grading 100-grade scale[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | p | Mean | SD | p | Mean | SD | p |
| Session I OI | 1.5 | ±0.92 | 0.549† | 2.0 | ±1.10 | 0.689† | 0.28 | ±0.14 | 0.168†† |
| Session II OI | 1.6 | ±0.87 | | 2.0 | ±1.20 | | 0.31 | ±0.18 | |
| Session I OII | 2.0 | ±0.91 | 0.424† | 2.6 | ±2.20 | 0.230† | 0.34 | ±0.14 | 0.218†† |
| Session II OII | 1.0 | ±0.89 | | 1.2 | ±1.00 | | 0.31 | ±0.12 | |
| Session I OIII | 1.6 | ±0.91 | 0.552† | 2.0 | ±1.08 | 0.424† | 0.30 | ±0.16 | |
| Session II OIII | 1.72 | ±1.08 | | 2.2 | ±1.21 | | 0.32 | ±0.19 | |

| (B) CI | Four-grade scale | Five-grade scale | Computerized grading 100-grade scale[a] |
|---|---|---|---|
| | Grade | Grade | Grade |
| OI: SI–SII | ±0.88 | ±0.99 | ±17.5 |
| OII: SI–SII | ±2.21 | ±2.00 | ±16.7 |
| OIII: SI–SII | ±1.31 | ±0.93 | ±16.8 |
| SII: OI–OII | ±1.76 | ±1.49 | ±12.8 |
| SII: OI–OIII | ±1.29 | ±0.91 | ±19.5 |
| SII: OII–OIII | ±1.31 | ±1.20 | ±26.9 |

| (C) Correl. | Four-grade scale | | Five-grade scale | | Computerized grading 100-grade scale[a] | |
|---|---|---|---|---|---|---|
| | r | p | r | p | r | p |
| OI: SI–SII | 0.88 | <0.001† | 0.91 | <0.001† | 0.88 | <0.001†† |
| OII: SI–SII | 0.16 | 0.214† | 0.58 | 0.001† | 0.81 | <0.001†† |
| OIII: SI–SII | 0.70 | <0.001† | 0.92 | <0.001† | 0.89 | <0.001† |
| SII: OI–OII | 0.50 | 0.005† | 0.61 | 0.005† | 0.79 | <0.001†† |
| SII: OI–OIII | 0.86 | <0.001† | 0.92 | <0.001† | 0.90 | <0.001†† |
| SII: OII–OIII | 0.54 | 0.003† | 0.73 | 0.004† | 0.71 | <0.001†† |

[a] Numbers of the computerized grading scale are the fraction of the tarsal area including the MG affected by MG loss.

**Table 2**
Cohens's weighted kappa statistic applied in subjective grading.

| κ-Statistic | Four-grade scale | | | Five-grade scale | | |
|---|---|---|---|---|---|---|
| | κ | p | 95% CI-κ | κ | p | 95% CI-κ |
| OI: SI–SII | 0.79 | <0.001 | 0.441; 1.000 | 0.80 | <0.001 | 0.500; 1.000 |
| OII: SI–SII | 0.15 | 0.342 | −0.164; 0.473 | 0.40 | 0.009 | 0.100; 0.700 |
| OIII: SI–SII | 0.50 | 0.007 | 0.136; 0.858 | 0.81 | <0.001 | 0.483; 1.000 |
| SII: OI–OII | 0.36 | 0.035 | 0.024; 0.689 | 0.60 | <0.001 | 0.292; 0.914 |
| SII: OI–OIII | 0.77 | <0.001 | 0.411; 1.000 | 0.90 | <0.001 | 0.600; 1.000 |
| SII: OII–OIII | 0.46 | 0.008 | 0.118; 0.800 | 0.57 | <0.001 | 0.252; 0.882 |

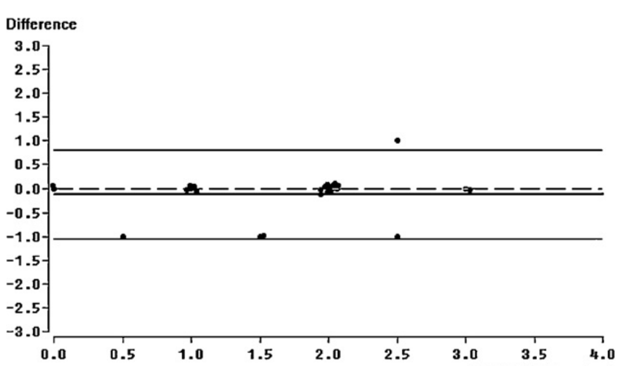


**Fig. 4.** Bland–Altman plot showing inter-observer agreement from grading of MG loss (four-grade scale (4S), OI–OIII; solid bolded bar shows mean difference, upper and lower horizontal lines represent 95% tolerance of differences).

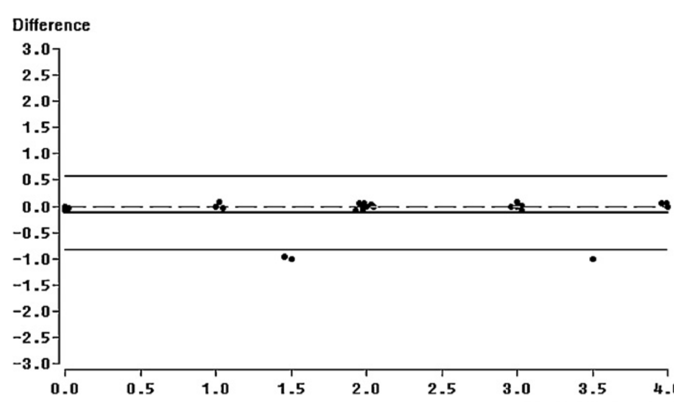


**Fig. 5.** Bland–Altman plot showing inter-observer agreement from grading of MG loss (five-grade scale (5S), OI–OIII; solid bolded bar shows mean difference, upper and lower horizontal lines represent 95% tolerance of differences).

## 4. Discussion

In this study, the repeatability of a five-grade scale was analysed in comparison to a four-grade scale [8]. Furthermore it was evaluated if computerized grading [14,16,23] improves the repeatability of the classification in meibography.

Intra-observer agreement (95% confidence intervals) of the five-grade scale was nearly similar to the four-grade scale in OI. But in OII and OIII the five-grade scale was better than the four-grade scale. When comparing the sessions, the correlation coefficients were not such different for both grading scales in OI and OIII.

**Table 3**
Conversion of CI degrees in percentage (% = CI degree × increment of scale) to facilitate comparison between scales (observer = O; session = S).

| CI | Four-grade scale | | Five-grade scale | | Digital analysis (100-grade scale) | |
|---|---|---|---|---|---|---|
| | Grade | % | Grade | % | Grade | % |
| OI: SI–SII | ±0.88 | >±22 | ±0.99 | ±25 | ±17.5 | ±18 |
| OII: SI–SII | ±2.21 | >±71 | ±2.00 | ±50 | ±16.7 | ±17 |
| OIII: SI–SII | ±1.31 | >±33 | ±0.93 | ±23 | ±16.8 | ±17 |
| SII: OI–OII | ±1.76 | >±44 | ±1.49 | ±37 | ±12.8 | ±13 |
| SII: OI–OIII | ±1.29 | >±32 | ±0.91 | ±23 | ±19.5 | ±20 |
| SII: OII–OIII | ±1.31 | >±33 | ±1.20 | ±30 | ±26.9 | ±26.9 |

However, sessions of OII were not correlated with the four-grade scale and even there was a significant correlation between sessions for OII when applying the five-grade scale the correlation coefficient was small only. For both subjective grading scales, the CI of OII was remarkably larger than that of the other observers. With the four-grade scale the intra-observer agreement of OI and OIII conformed published figures [8]. Even though all were experienced observers, there could be disagreement between observers and indicate the magnitude of this even though there was no detectable statistical significance.

Nevertheless, the kappa statistics showed fair to substantial reliability of the five-grade scale while it was slight to moderate only for the four-grade scale. $\kappa$ Values are classified for reference as follows: 0.00 (poor reliability), 0.00–0.20 (slight reliability), 0.21–0.40 (fair reliability), 0.41–0.60 (moderate reliability), 0.61–0.80 (substantial reliability), and greater than 0.8 (close to perfect reliability) [27].

The five-grade scale might be superior to the four-grade scale for three reasons: firstly, there was a better intra-observer repeatability of the five-grade scale for the observers OII and OIII. Secondly, inter-observer agreement was notably better with the five-grade scale. Thirdly, the five-grade scale gave more consistent increments (Fig. 6). When evaluating changes of meibomian gland morphology it might be difficult to transform the non-linear increments of the four-grade scale in percentage change. This can be more successfully achieved with the five-grade scale, making it more comparable to other subjective grading scales and computerized analyses of MGL. Furthermore, finer increments may enhance the detection of cut-off values and changes of meibomian gland morphology (Fig. 7).

However, the intra-observer and inter-observer agreement, especially for OII, shows the limitation of subjective grading. Conversion of the CI intervals of the subjective grading scales into percentage to compare agreement with the computerized grading clearly shows the advantage of computerized grading in meibography (Table 3). The intra-observer agreement and inter-observer agreement was much better with applied computerized grading than with the subjective grading scales and this approach may be promising in future research projects. The applied computerized grading [14,16,20,23] represents a semi-objective method. Calculating the ratio of the total area and area of loss may reduce this limitation. A more standardized and automatized method may improve repeatability and precision [28].

Even though agreement between sessions and observers was notably better with computerized classification, digital analyses of images applying ImageJ software may be too time consuming in the daily routine of clinical practice. Using the five-grade scale in the classification of the meibomain glands may represent an alternative option. Nevertheless, implementation of future automatized and user friendly digital analyses software may promote the application of meibography in clinical practice.

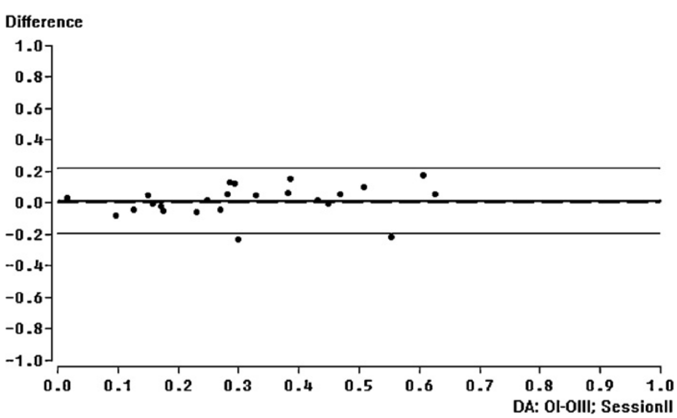


**Fig. 6.** Bland–Altman plot showing inter-observer agreement from computerized grading of MG loss by digital image analyses (DA) (OI–OIII; solid bolded bar shows mean difference, upper and lower horizontal lines represent 95% tolerance of differences).

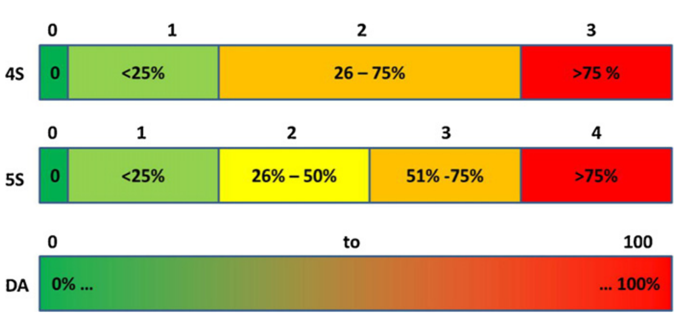


**Fig. 7.** Comparison of the increments of the evaluated scales.

## 5. Conclusion

Better linearity and finer increments of the five-grade scale may improve subjective grading. However, intra-observer and inter-observer agreement was better with computerized grading followed by the subjective five-grade scale and the subjective four-grade scale. Computerized grading of the meibomian glands should have a promising future.

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