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# Repeatability of Meibomian Gland Contrast, a Potential Indicator of Meibomian Gland Function

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**Purpose:** Meibomian gland contrast may be a potential indicator of gland health, especially among isotretinoin users. We aimed to develop a repeatable and reliable method for measuring Meibomian gland contrast from meibography images.

**Methods:** Lower lid (LL) and upper lid (UL) meibography were captured with the OCULUS Keratograph 5M (OCULUS, Inc) at 2 visits under the following 4 conditions: face centered with room lights on (C), left-turned face (L), right-turned face (R), and face centered with room lights off (CLO). Contrast was measured with Fiji (v2.0.0-rc-59). Coefficient of repeatability and limits of agreement (LOA) were determined using Bland-Altman plots.

**Results:** A total of 512 meibography images from 16 subjects (age ± SD = 24.8 ± 5.2 years; 13 female patients) were collected. Coefficient of repeatability between visits was 10.5 for UL and 14.9 for LL. Lower and upper LOA, respectively, for UL, compared with condition C, were −10.9 [95% confidence interval (CI), −13.5 to −8.3] and 6.2 (95% CI, 3.6–8.8) for L; −11.0 (95% CI, −13.8 to −8.1) and 7.0 (95% CI, 4.2–9.8) for R; and −9.0 (95% CI, −11.6 to −6.5) and 7.2 (95% CI, 4.7–9.8) for CLO. Lower and upper LOA, respectively, for LL, compared with condition C, were −18.1 (95% CI, −22.6 to −13.5) and 11.0 (95% CI, 6.5–15.5) for L; −15.3 (95% CI, −19.2 to −11.3) and 9.9 (95% CI, 6.0–13.9) for R; and −12.0 (95% CI, −15.1 to −8.8) and 8.2 (95% CI, 5.0–11.3) for CLO.

**Conclusions:** Meibomian gland contrast is a repeatable and reliable measure for changes in Meibomian gland contrast greater than 11 in the UL and 18 in the LL.

**Key Words:** tear lipid layer, meibomian gland, tear film stability, meibomian gland expressibility, meibography, evaporative dry eye, meibomian gland dysfunction, meibomian gland contrast, isotretinoin, 13-*cis*-retinoic acid, accutane, contrast, meibomian gland intensity, intensity, dry eye, dry eye disease, repeatability, limits of agreement

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Meibomian gland dysfunction (MGD) is believed to be the most common cause of ocular dryness symptoms.[1] Extensive effort has been made to understand the pathophysiology of MGD-induced evaporative dry eye, particularly the relationship between Meibomian gland dropout and meibum output in vivo. Meibomian gland dropout implies partial or total gland loss or atrophy, and it has been estimated by meiboscopy and meibography. Meiboscopy allows visualization of Meibomian glands by retroillumination of the eyelids, usually with a penlight or transilluminator, whereas meibography also includes photo-documentation. More recent meibography innovations include biomicroscopes and corneal topographers equipped with infrared cameras that produce images with better contrast between the Meibomian glands and surrounding tarsal plate and tissues. More advanced systems integrate both retroillumination and infrared photo-documentation to take advantage of both methods.

When evaluating meibography images, we make the assumptions that the glands, which appear as bright linear structures on meibography, are supposed to extend the full length of the tarsal plate, and when they do not, they are assumed to have functional gland loss, or atrophied. The degree of atrophy is most commonly assessed as a percentage of gland loss area (eg, space unoccupied by Meibomian glands) compared with the presumed full area of the tarsal plate. The estimated percentage can then be assigned a grade using one of several ordinal scales to represent severity.[2–8] Although grading atrophy is useful, especially in cases like obstructive MGD in which glands appear shorter because of hyporeflectivity proximal to the blockage, it may not be as useful in cases of hyposecretory MGD, where a global suppression of meibum production may result in overall dimming or fading of whole glands, not just shortening. In such instances, we would expect to see decreased intensity of all Meibomian glands along their full lengths but would be unable to characterize them using the existing Meibomian gland atrophy grading system.

In this study, we aim to demonstrate that measuring contrast in the region of the central 5 Meibomian glands from meibography images captured and processed with the OCULUS Keratograph 5M is repeatable between visits and show good agreement between different head positions and room lighting conditions. Having an objective, reliable, and repeatable grading method can be valuable for detecting subtle changes in meibography because of age, disease, or intervention, particularly when Meibomian gland length may not change.

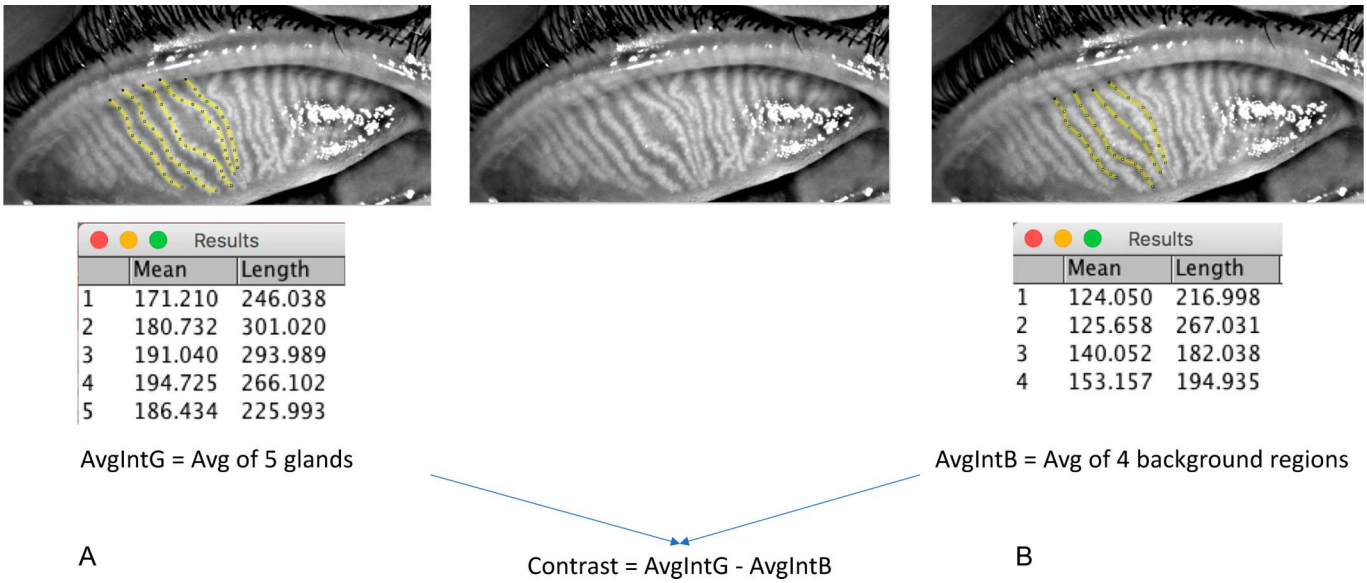



**FIGURE 1.** Contrast is the difference in mean pixel intensity of (A) central 5 glands (AvgIntG) (yellow lines represent glands; arrows point to leftmost measured gland) and (B) background Intensity between glands (AvgIntB) (yellow lines represent background space; arrows point to space between the 2 leftmost glands). AvgIntG, average of 5 glands; AvgIntB, average of 4 background regions.

## METHOD

### Subjects

Study participants were recruited from the University of California, Berkeley campus and surrounding community, and came for 2 visits at the Clinical Research Center in the School of Optometry. Participants were required to be 18 years or older and free of ocular infection, inflammation, or disease and systemic disease. Participants were excluded if using oral or ophthalmic medications and if their medical history changed between visits. Written informed consent was obtained from all study participants, and the study adhered to the tenets of the Declaration of Helsinki. The study protocol was approved by the University of California, Berkeley, Office for Protection of Human Subjects.

### Meibography Images

Meibography of upper (UL) and lower (LL) eyelids from both eyes of study participants were captured with the OCULUS Keratograph 5M (OCULUS, Inc, Arlington, WA), which produces 2 images: raw and processed. The OCULUS-processed images have increased contrast between the Meibomian glands and surrounding tissues and are the ones analyzed in this study. Meibography was captured at 2 separate visits under the following 4 conditions: face centered with room lights on, face turned left, face turned right, and face centered with room lights off. Using Fiji (version 2.0.0-rc-59/1.51k),[9] an image processing package (ImageJ with plugins), mean pixel intensity (gray scale: 0–255) was measured of segmented lines drawn along the central 5 Meibomian glands (Fig. 1A) and along the background regions between the Meibomian glands measured (Fig. 1B). The difference between mean intensity along the Meibomian glands and mean intensity along background regions between the MGs was defined as contrast.

### Statistical Methods

Using previously published methods, the sample size was estimated to be 13 study participants, with 16 UL and 16 LL measurements per participant over 2 visits.[10] The coefficient of repeatability was measured for the same measurement conditions between visits, and the limits of agreement (LOA) for face turned left, face turned right, and face centered with room lights off when each are compared with face centered with room lights on were determined using Bland-Altman plots.[11]

## RESULTS

### Subjects

Meibography images of 16 subjects (age ± SD = 24.8 ± 5.2 years) were collected over 2 visits (separated by 1–4 days) under 4 different conditions for both upper and lower eyelids of

**TABLE 1.** Mean Contrast for Each Test Condition at Each Visit

| | Visit 1 | Visit 2 |
|---|---|---|
| Face centered with room lights on | | |
| Upper lid | 40.8 ± 12.2 | 40.7 ± 12.9 |
| Lower lid | 60.1 ± 19.1 | 61.4 ± 20.9 |
| Face turned left | | |
| Upper lid | 39.1 ± 12.0 | 37.7 ± 12.3 |
| Lower lid | 55.6 ± 20.8 | 58.8 ± 22.1 |
| Face turned right | | |
| Upper lid | 39.3 ± 14.1 | 38.2 ± 13.5 |
| Lower lid | 57.0 ± 18.7 | 59.1 ± 20.6 |
| Face centered with room lights off | | |
| Upper lid | 39.0 ± 11.0 | 40.7 ± 12.9 |
| Lower lid | 57.7 ± 18.5 | 60.0 ± 18.3 |

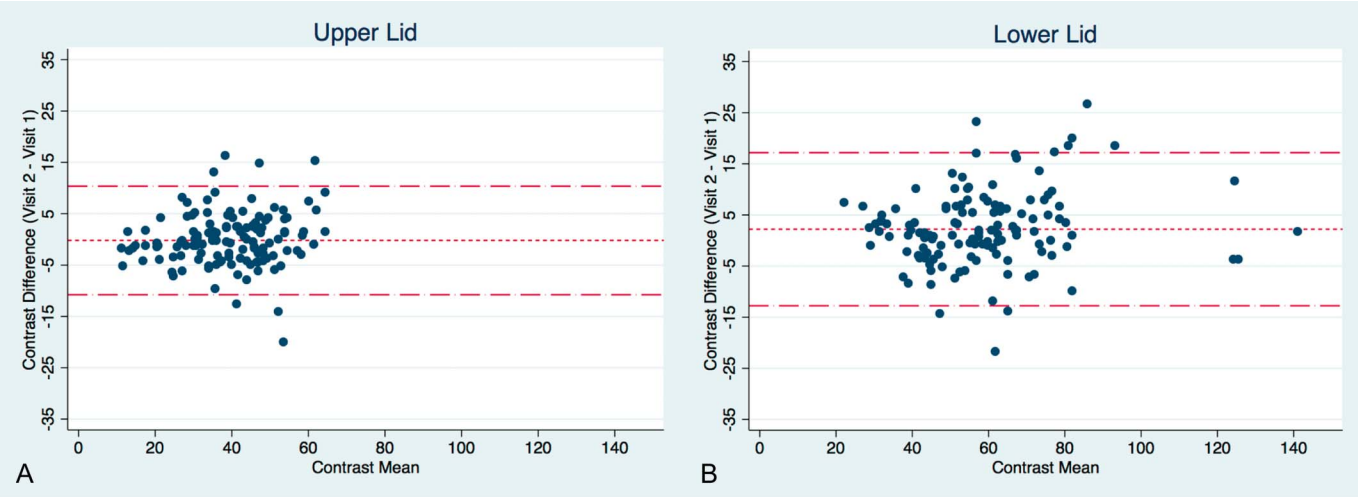



**FIGURE 2.** Contrast differences versus means plots comparing visit 1 to visit 2 for (A) upper lid and (B) lower Lid.

both eyes, totaling 512 images. The study population included 13 female patients and 14 Asians.

## Repeatability

The mean (±SD) contrast for the LL was consistently higher than that for the UL (Table 1). Comparing measurements taken between visits, the average differences in contrast between visits and coefficients of repeatability, respectively, were −0.21 ± 5.28 and 10.53 for the upper eyelid, 2.20 ± 7.48 and 14.91 for the lower eyelids, and −1.00 ± 6.58 and 13.13 when combining both upper and lower eyelids. The differences versus means plots for the upper and lower eyelids are presented in Figure 2. In general, the contrast measurement exhibited best repeatability with the upper eyelid meibography images compared with the LL meibography images.

## Limits of Agreement

The mean Meibomian gland and background intensities as well as mean contrast for each lid under each of the 4 previously defined conditions are reported in Table 2. Using the centered position with room lights on as the reference, mean differences in Meibomian gland contrast were estimated against other head positions/room conditions for the upper eyelid, lower eyelid, and both lids combined. The differences versus means plots for these comparisons are presented in Figure 3. In general, the mean differences in contrast were lower for the upper eyelid than for the lower eyelid.

For the upper eyelid, the lower and upper LOA compared with the reference condition were −10.9 [95% confidence interval (CI): −13.5 to −8.3] and 6.2 (95% CI, 3.6–8.8), respectively, for left-turned faces; −11.0 (95% CI, −13.8 to −8.1) and 7.0 (95% CI, 4.2–9.8), respectively, for

**TABLE 2.** Mean Meibomian Gland Intensity, Background Intensity, and Contrast for Each Eyelid Under Each Test Condition

| | Upper Eyelid | | Lower Eyelid | |
|---|---|---|---|---|
| | Mean ± SD | Range | Mean ± SD | Range |
| Center | | | | |
| Gland intensity | 187.5 ± 17.7 | 157.9–223.7 | 189.6 ± 18.3 | 152.4–220.7 |
| Background intensity | 146.7 ± 12.9 | 123.7–173.8 | 129.5 ± 24.6 | 44.9–171.8 |
| Contrast | 40.7 ± 12.4 | 15.0–64.5 | 60.7 ± 19.6 | 29.3–125.9 |
| Left | | | | |
| Gland intensity | 181.8 ± 18.3 | 144.7–215.9 | 186.6 ± 15.9 | 158.1–216.9 |
| Background intensity | 142.7 ± 17.3 | 96.9–183.4 | 130.9 ± 24.1 | 47.6–179.0 |
| Contrast | 38.4 ± 11.9 | 11.6–58.5 | 57.2 ± 21.3 | 27.4–141.4 |
| Right | | | | |
| Gland intensity | 182.6 ± 19.6 | 143.4–216.3 | 186.5 ± 17.3 | 150.7–219.0 |
| Background intensity | 143.3 ± 14.2 | 116.5–172.6 | 129.5 ± 23.0 | 50.9–177.4 |
| Contrast | 38.8 ± 13.4 | 13.5–64.6 | 58.1 ± 19.2 | 22.3–124.8 |
| Center–room lights off | | | | |
| Gland intensity | 181.0 ± 15.7 | 154.2–217.6 | 187.7 ± 19.5 | 149.5–223.3 |
| Background intensity | 142.0 ± 14.8 | 113.7–171.4 | 130.0 ± 28.9 | 23.1–174.4 |
| Contrast | 39.8 ± 11.7 | 11.8–61.9 | 58.8 ± 18.0 | 29.1–124.4 |

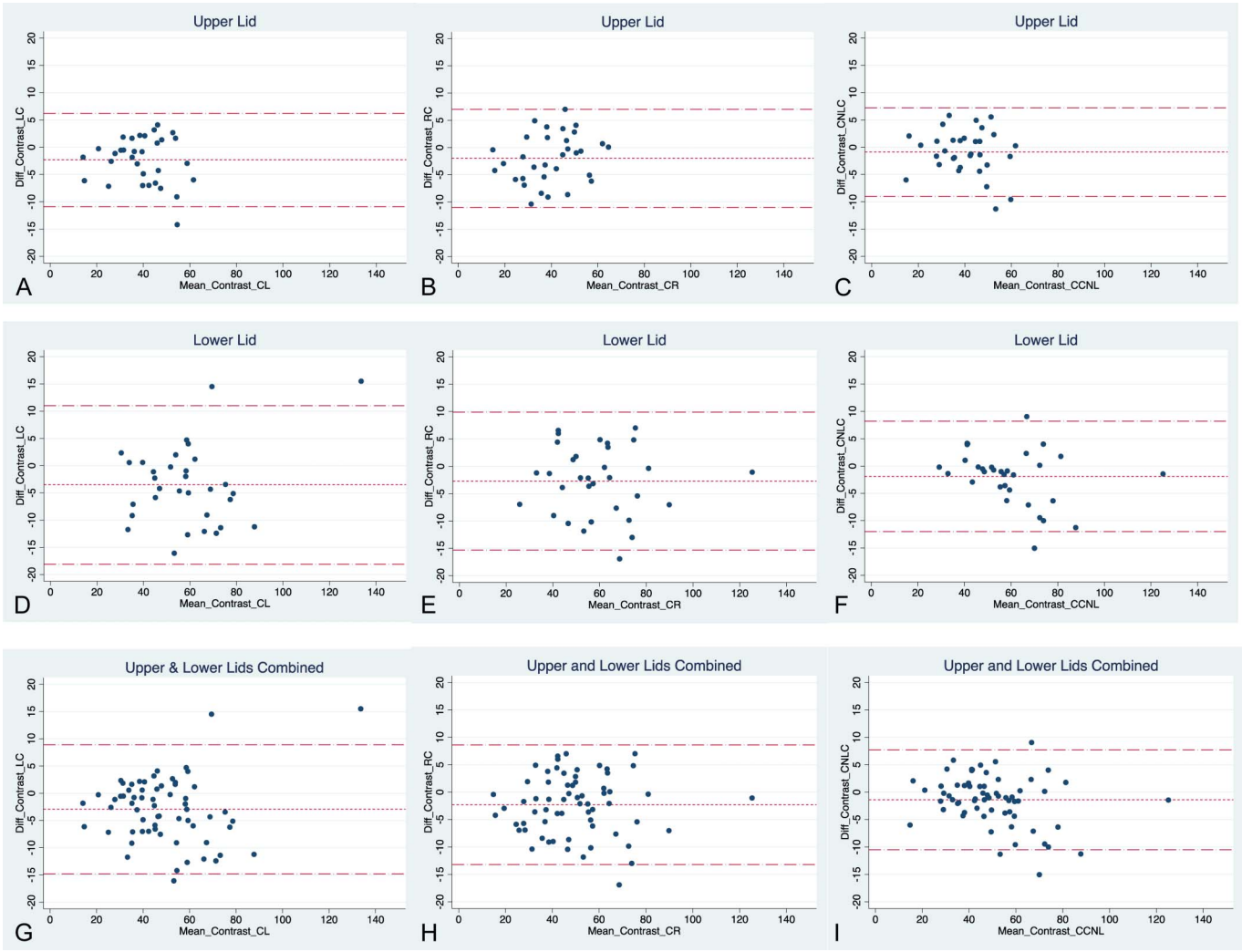



**FIGURE 3.** Contrast differences versus means plots for the upper eyelids (A–C), lower eyelids (D–F), and upper and lower lids combined (G–I). Plots comparing left against centered head positions are (A), (D), and (G); right against centered head positions are (B), (E), and (H); and centered head positions with room lights off against room lights on are (C), (F), and (I).

right-turned faces; and −9.0 (95% CI, −11.6 to −6.5) and 7.2 (95% CI, 4.7–9.8), respectively, for centered faces with room lights off (Table 3). For the lower eyelid, the lower and upper LOA compared with the reference condition were −18.1 (95% CI, −22.6 to −13.5) and 11.0 (95% CI, 6.5–15.5), respectively, for left-turned faces; −15.3 (95% CI, −19.2 to −11.3) and 9.9 (95% CI, 6.0–13.9), respectively, for right-turned faces; and −12.0 (95% CI, −15.1 to −8.8) and 8.2 (95% CI, 5.0–11.3), respectively, for centered faces with room lights off. When both upper and lower eyelids were combined, the lower and upper LOA compared with the reference condition were −14.8 (95% CI, −17.4 to −12.2) and 8.9 (95% CI, 6.4–11.5), respectively, for left-turned faces; −13.2 (95% CI, −15.5 to −10.8) and 8.6 (95% CI, 6.2–10.9), respectively, for right-turned faces; and −10.5 (95% CI, −12.5 to −8.6) and 7.7 (95% CI, 5.8–9.7), respectively, for centered faces with room lights off.

## DISCUSSION

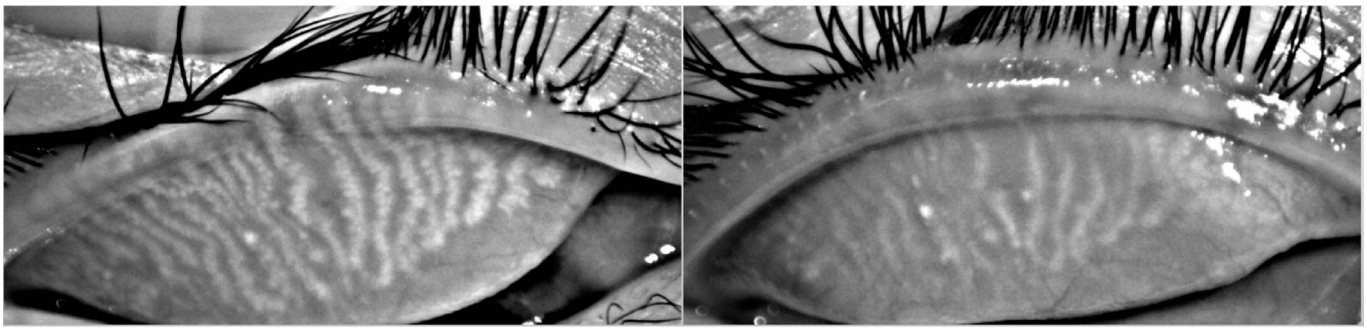
This study aimed to determine the repeatability and reliability of measuring meibography contrast of the central-5-gland region of both upper and lower eyelids. Measurements were taken at 2 separate visits to determine repeatability and under 4 different conditions for both eyes to estimate the LOA. We found that the coefficient of repeatability was 10.5 for the upper eyelid and 14.9 for the lower eyelid, when comparing images taken under same conditions but at different visits. Furthermore, the LOA for the upper eyelid were similar when comparing centered head position to left (−10.9 to 6.2) or right (−11.0 to 7.0) head positions and were smallest compared with centered head position with room lights off (−9.0 to 7.2). These results suggest that 95% of individuals will have a difference in contrast in the upper eyelid between approximately −11.0 and 7.2, at the most, based on the extreme values of the UL LOA. For the lower eyelid, the LOA were all further apart than those for the upper

**TABLE 3.** Limits of Agreement (LOA, Matched by Visit) for Upper Lids, Lower Lids, and all Lids

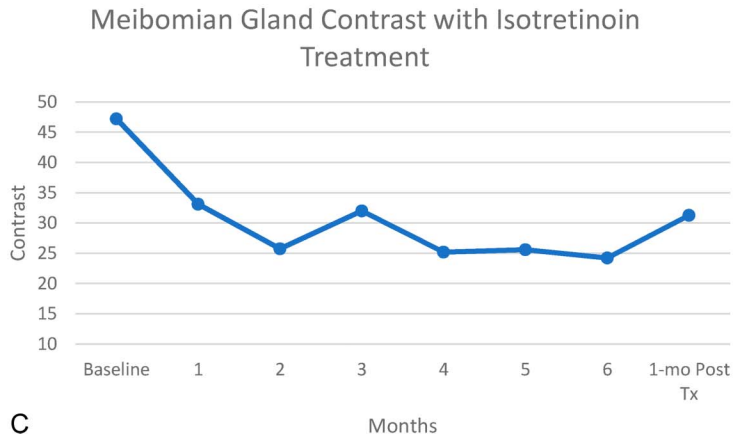
| | Left Versus Center With Lights On | | Right Versus Center With Lights On | | Center With Lights Off Versus Center With Lights On | |
|---|---|---|---|---|---|---|
| | Lower LOA | Upper LOA | Lower LOA | Upper LOA | Lower LOA | Upper LOA |
| Upper lid | | | | | | |
| LOA | −10.9 | 6.2 | −11.0 | 7.0 | −9.0 | 7.2 |
| SE | 1.3 | 1.3 | 1.4 | 1.4 | 1.2 | 1.2 |
| 95% CI | −13.5 to −8.3 | 3.6 to 8.8 | −13.8 to −8.1 | 4.2 to 9.8 | −11.6 to −6.5 | 4.7 to 9.8 |
| Lower lid | | | | | | |
| LOA | −18.1 | 11.0 | −15.3 | 9.9 | −12.0 | 8.2 |
| SE | 2.2 | 2.2 | 1.9 | 1.9 | 1.5 | 1.5 |
| 95% CI | −22.6 to −13.5 | 6.5 to 15.5 | −19.2 to −11.3 | 6.0 to 13.9 | −15.1 to −8.8 | 5.0 to 11.3 |
| Combined lids | | | | | | |
| LOA | −14.8 | 8.9 | −13.2 | 8.6 | −10.5 | 7.7 |
| SE | 1.3 | 1.3 | 1.2 | 1.2 | 1.0 | 1.0 |
| 95% CI | −17.4 to −12.2 | 6.4 to 11.5 | −15.5 to −10.8 | 6.2 to 10.9 | −12.5 to −8.6 | 5.8 to 9.7 |

eyelid, and gap was greatest when comparing the centered head position with left (−18.1 to 11.0) or right (−15.3 to 9.9) head positions and was smaller compared with centered head position with room lights off (−12.0 to 8.2). Based on the extreme values of the LL LOA, the results suggest that 95% of individuals will have a difference in contrast in the LL between approximately −18.1 and 8.2, at the most. When the data for both upper and lower eyelids were combined, the LOA were furthest apart when comparing the centered head position with the left head position (−14.8 to 8.9) and right head position (−13.2 to 8.6) and were closest compared with the centered head position with room lights off (−10.5 to 7.7).

It is unclear what is exactly seen on meibography images (measured at 840 nm), but we know that many organic compounds, such as lipids, are highly reactive to infrared light. This is the basis for infrared spectroscopy, which uses medium infrared wavelengths to produce



A B




C

**FIGURE 4.** Isotretinoin patient upper lid meibography images at (A) baseline and (B) after 6 months of treatment, and (C) contrast Changes during treatment.

qualitative information on functional groups used to identify compounds.[12] Infrared imaging is also used to visualize subretinal lipid deposits, such as drusen, at wavelengths greater than 800 nm.[13] Hartnett and Elsner[14] found that IR imaging at 865 nm of the retina belonging to patients with exudative age-related macular degeneration provided the best visualization of drusen, as well as numerous other subretinal deposits that were not apparent clinically or through other methods such as fluorescein angiography and indocyanine green angiography. With respect to meibography, the consensus is that the highly reflective linear structures represent lipid-filled Meibomian gland ducts connected by ductules to acini containing lipid-producing meibocytes. It is unclear whether the reflectivity of the presumed glands is an indicator of gland function, but the case presented in Figure 4 suggests that it may, in fact, be true. Figure 4 presents a case belonging to a 19-year-old Asian male patient who received a course of isotretinoin treatment. Images were taken before commencing treatment (Fig. 4A) and after 5 months of treatment (Fig. 4B). It is notable that the reflectivity of the glands, assessed using the contrast measurement described in this article, decreased during treatment and then increased after discontinuing treatment. It is interesting to note that the length of the glands remained fairly constant throughout, so measuring percent atrophy would have overlooked an important change occurring inside the glands. Studies have shown that isotretinoin shrinks human sebaceous glands, increases the presence of undifferentiating cells, and inhibits sebum production.[15,16] In relation to immortalized human Meibomian gland epithelial cells, 13-*cis*-retinoic acid was shown to increase cell death and inhibits cell proliferation.[17] Therefore, the decreased reflectivity of the Meibomian glands, especially in isotretinoin cases, may be an indication of shrinking meibocytes, decreased cell proliferation, and, as a result, decreased meibum production.

In summary, measuring the contrast in the central-5-gland region of meibography images is a repeatable and reliable method for potentially tracking longitudinal changes to Meibomian glands because of age, disease, or intervention, particularly when systemic effects are expected. Contrast changes greater than 11 units in the upper eyelid or 18 units in the lower eyelid are less likely because of head position, room lighting, or inherent variations, but would more likely be because of physiologic changes within the Meibomian glands. As evidenced by the isotretinoin case in Figure 4, contrast can be useful in monitoring patients using other medications known to be associated with MGD, including antidepressants/antipsychotics, antiandrogens, and antihistamines.[18–23] It would also be beneficial in identifying changes that may occur with diseases known to be associated with MGD, such as androgen deficiency, atopy, psoriasis, and rosacea.[21,22,24–27]

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