Published in final edited form as:

Cornea. 2019 February ; 38(2): 256–261. doi:10.1097/ICO.0000000000001818.

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

Thao N. Yeh, OD, MPH[1,2] and **Meng C. Lin, OD, PhD**[1,2]

[1]Clinical Research Center, School of Optometry, University of California, Berkeley, USA

[2]Vision Science Group, School of Optometry, University of California, Berkeley, USA

Abstract

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

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

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

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

Keywords

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

---

Corresponding Author: Thao N. Yeh, University of California, Berkeley, School of Optometry 360 Minor Hall Berkeley, CA 94720-2020, Phone: 510-642-9649, Fax: 510-642-9734, thaoyeh@berkeley.edu.

Commercial relationship interest: None (TNY and MCL)

The authors have no conflicts of interest associated with this study.

Corresponding author is different from the author who is to receive reprints.

INTRODUCTION

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, while 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 the surrounding tarsal plate and tissues. More advanced systems integrate both retroillumination and infrared photodocumentation 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 (e.g., space unoccupied by Meibomian glands) compared to 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]. While grading atrophy is useful, especially in cases like obstructive MGD in which glands appear shorter due to hypo-reflectivity 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 those 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 five 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 due to age, disease, or intervention, particularly when Meibomian gland length may not change.

METHOD

Subjects

Study participants were recruited from the University of California, Berkeley campus and surrounding community, and came for two 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 and lower eyelids from both eyes of study participants were captured with the OCULUS Keratograph 5M (OCULUS, Inc., Arlington, WA), which produces two images: raw and processed. The OCULUS-processed images have increased contrast between the Meibomian glands and the surrounding tissues and are the ones analyzed in this study. Meibography was captured at two separate visits under four conditions: face centered with 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 (greyscale: 0–255) was measured of segmented lines drawn along the central 5 meibomian glands (Figure 1a) and along the background regions between the Meibomian glands measured (Figure 1b). The difference between mean intensity along the Meibomian glands and the 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 upper lid and 16 lower lid measurements per participant over two 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 lights off when each are compared to face centered with lights on were determined using Bland-Altman plots.[11]

RESULTS

Subjects

Meibography images of 16 subjects [age±standard deviation (SD) = 24.8±5.2 years] were collected over two visits (separated by 1–4 days) under four different conditions for both upper and lower eyelids of both eyes, totaling 512 images. The study population included 13 females and 14 Asians.

Repeatability

The mean (± SD) contrast for the lower lid was consistently higher than that of the upper lid (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 the upper and lower eyelids. The Differences vs. Means plots for the upper eyelid and lower eyelid are presented in Figure 2. In general, the contrast measurement exhibited best repeatability with the upper eyelid meibography images compared to the lower lid.

Limits of Agreement

The mean Meibomian gland and background intensities, as well as mean contrast for each lid under each of the four previously defined conditions are reported in Table 2. Using the centered position with 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 difference vs. mean plots for these comparisons are presented in Figure 3. In general, the mean differences in contrast were lower for the upper eyelid compared to the lower eyelid.

For the upper eyelid, the lower and upper limits of agreement when compared to the reference condition were −10.9 (95% CI: −13.5, −8.3) and 6.2 (95% CI: 3.6, 8.8), respectively, for left-turned faces; −11.0 (95% CI: −13.8, −8.1) and 7.0 (95% CI: 4.2, 9.8), respectively, for right-turned faces; and −9.0 (95% CI: −11.6, −6.5) and 7.2 (95% CI: 4.7, 9.8), respectively, for centered faces with lights off (Table 3). For the lower eyelid, the lower and upper limits of agreement when compared to the reference condition were −18.1 (95% CI: −22.6, −13.5) and 11.0 (95% CI: 6.5, 15.5), respectively, for left-turned faces; −15.3 (95% CI: −19.2, −11.3) and 9.9 (95% CI: 6.0, 13.9), respectively, for right-turned faces; and −12.0 (95% CI: −15.1, −8.8) and 8.2 (95% CI: 5.0, 11.3), respectively, for centered faces with lights off. When both the upper and lower eyelids were combined, the lower and upper limits of agreement when compared to the reference condition were −14.8 (95% CI: −17.4, −12.2) and 8.9 (95% CI: 6.4, 11.5), respectively, for left-turned faces; −13.2 (95% CI: −15.5, −10.8) and 8.6 (95% CI: 6.2, 10.9), respectively, for right-turned faces; and −10.5 (95% CI: −12.5, −8.6) and 7.7 (95% CI: 5.8, 9.7), respectively, for centered faces with lights off.

DISCUSSION

This study aimed to determine the repeatability and reliability of measuring meibography contrast of the central-5-gland region of both the upper and lower eyelids. Measurements were taken at two separate visits to determine repeatability and under 4 different conditions for both eyes to estimate the limits of agreement. 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 limits of agreement for the upper eyelid were similar when comparing centered head position to left (−10.9, 6.2) or right (−11.0, 7.0) head positions and was smallest when compared with centered head position with lights off (−9.0, 7.2). These results suggest that 95% of individuals will have a difference in contrast in the upper lid between approximately −11.0 and 7.2, at the most, based on the extreme values of the upper lid limits of agreement. For the lower eyelid, the limits of agreement were all further apart than those of the upper eyelid and gap was greatest when comparing the centered head position with left (−18.1, 11.0) or and right (−15.3, 9.9) head positions and was smaller when compared with centered head position with lights off (−12.0, 8.2). Based on the extreme values of the lower lid limits of agreement, the results suggest that 95% of individuals will have a difference in contrast in the lower lid between approximately - 18.1 and 8.2, at the most. When the data for both upper and lower eyelids were combined, the limits of agreement were furthest apart when comparing the centered head position with the left head position (−14.8, 8.9) and right head position (−13.2, 8.6), and was closest when compared with the centered head position with lights off was (−10.5, 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 qualitative information on functional groups used to identify compounds.[12] Infrared imaging is also used to visualize sub-retinal lipid deposits, such as drusen, at wavelengths greater than 800 nm.[13] Hartnett, et al., 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 sub-retinal deposits that were not apparent clinically or through other methods such as fluorescein angiography and indocyanine green angiography.[14] 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 if the reflectivity of the presumed glands is an indicator of gland function, but the case presented in Figure 4 suggests that that may, in fact, be true. Figure 4 presents a case belonging to a 19-year-old Asian male who received a course of isotretinoin treatment. Images were taken prior to commencing treatment (4a) and after five months of treatment (4b). It is notable that the reflectivity of the glands, assessed using the contrast measurement described in this paper, 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 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 due to 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 due to head position, room lighting, or inherent variations, but would more likely be due to physiological 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 Meibomian gland dysfunction, 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 Meibomian gland dysfunction, such as androgen deficiency, atopy, psoriasis, and rosacea.[21,22,24–27]

Acknowledgments

Grant/Financial Support: NIH K23EY02665

REFERENCES

1. Schaumberg DA, Nichols JJ, Papas EB, et al. The international workshop on meibomian gland dysfunction: report of the subcommittee on the epidemiology of, and associated risk factors for, MGD. Invest Ophthalmol Vis Sci. 2011;52:1994–2005. [PubMed: 21450917]

2. Nichols JJ, Jones L, Nelson JD, et al. The TFOS International Workshop on Contact Lens Discomfort: introduction. Invest Ophthalmol Vis Sci. 2013;54:TFOS1–6. [PubMed: 24058136]
3. Mathers WD, Shields WJ, Sachdev MS, et al. Meibomian gland dysfunction in chronic blepharitis. Cornea. 1991;10:277–285. [PubMed: 1889213]
4. 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. [PubMed: 9436879]
5. Jester J V, Nicolaides N, Smith RE. Meibomian gland dysfunction. I. Keratin protein expression in normal human and rabbit meibomian glands. Invest Ophthalmol Vis Sci. 1989;30:927–935. [PubMed: 2470693]
6. Shimazaki J, Sakata M, Tsubota K. Ocular surface changes and discomfort in patients with meibomian gland dysfunction. Arch Ophthalmol (Chicago, Ill 1960). 1995;113:1266–1270.
7. Arita R, Itoh K, Inoue K, et al. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. Ophthalmology. 2008;115:911–915. [PubMed: 18452765]
8. de Paiva CS, Lindsey JL, Pflugfelder SC. Assessing the severity of keratitis sicca with videokeratoscopic indices. Ophthalmology. 2003;110:1102–1109. [PubMed: 12799233]
9. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological- image analysis. Nat Meth. 2012;9:676–682.
10. McAlinden BCh CM, Khadka J, Pesudovs K. Precision (repeatability and reproducibility) studies and sample-size calculation. J Cart Refract Surg. 2015;41:2598–2604.
11. Martin Bland J, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;327:307–310.
12. Cast J Infrared spectroscopy of lipids In: Developments in Oils and Fats. Boston, MA: Springer US; 1995:224–266.
13. Ly A, Nivison-Smith L, Assaad N, et al. Infrared reflectance imaging in age-related macular degeneration. Ophthalmic Physiol Opt. 2016;36:303–316. [PubMed: 27112225]
14. Hartnett ME, Elsner AE. Characteristics of exudative age-related macular degeneration determined in vivo with confocal and indirect infrared imaging. Ophthalmology. 1996;103:58–71. [PubMed: 8628562]
15. Nelson AM, Gilliland KL, Cong Z, et al. 13-cis Retinoic Acid Induces Apoptosis and Cell Cycle Arrest in Human SEB-1 Sebocytes. J Invest Dermatol. 2006;126:2178–2189. [PubMed: 16575387]
16. Zouboulis CC. Isotretinoin Revisited: Pluripotent Effects on Human Sebaceous Gland Cells. J Invest Dermatol. 2006;126:2154–2156. [PubMed: 16983322]
17. Ding J, Kam WR, Dieckow J, et al. The Influence of 13-cis Retinoic Acid on Human Meibomian Gland Epithelial Cells. Invest Ophthalmol Vis Sci. 2013;54:4341–4350. [PubMed: 23722388]
18. Chia E-M, Mitchell P, Rochtchina E, et al. Prevalence and associations of dry eye syndrome in an older population: the Blue Mountains Eye Study. Clin Experiment Ophthalmol. 2003;31:229–232. [PubMed: 12786773]
19. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. Arch Ophthalmol. 2000;118:1264–1268. [PubMed: 10980773]
20. Schaumberg DA, Dana R, Buring JE, et al. Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. Arch Ophthalmol. 2009;127:763–768. [PubMed: 19506195]
21. Krenzer KL, Dana MR, Ullman MD, et al. Effect of androgen deficiency on the human meibomian gland and ocular surface. J Clin Endocrinol Metab. 2000;85:4874–4882. [PubMed: 11134156]
22. Sullivan BD, Evans JE, Krenzer KL, et al. Impact of antiandrogen treatment on the fatty acid profile of neutral lipids in human meibomian gland secretions. J Clin Endocrinol Metab. 2000;85:4866–4873. [PubMed: 11134155]
23. Sullivan DA, Sullivan BD, Evans JE, et al. Androgen deficiency, Meibomian gland dysfunction, and evaporative dry eye. Ann N Y Acad Sci. 2002;966:211–222. [PubMed: 12114274]
24. Bron AJ, Benjamin L, Snibson GR. Meibomian gland disease. Classification and grading of lid changes. Eye (Lond). 1991;5 (Pt 4):395–411. [PubMed: 1743355]

25. Sullivan DA, Sullivan BD, Ullman MD, et al. Androgen influence on the meibomian gland. Invest Ophthalmol Vis Sci. 2000;41:3732–3742. [PubMed: 11053270]
26. Zengin N, Tol H, Balevi S, et al. Tear film and meibomian gland functions in psoriasis. Acta Ophthalmol Scand. 1996;74:358–360. [PubMed: 8883550]
27. Zengin N, Tol H, Gündüz K, et al. Meibomian gland dysfunction and tear film abnormalities in rosacea. Cornea. 1995;14:144–146. [PubMed: 7743796]

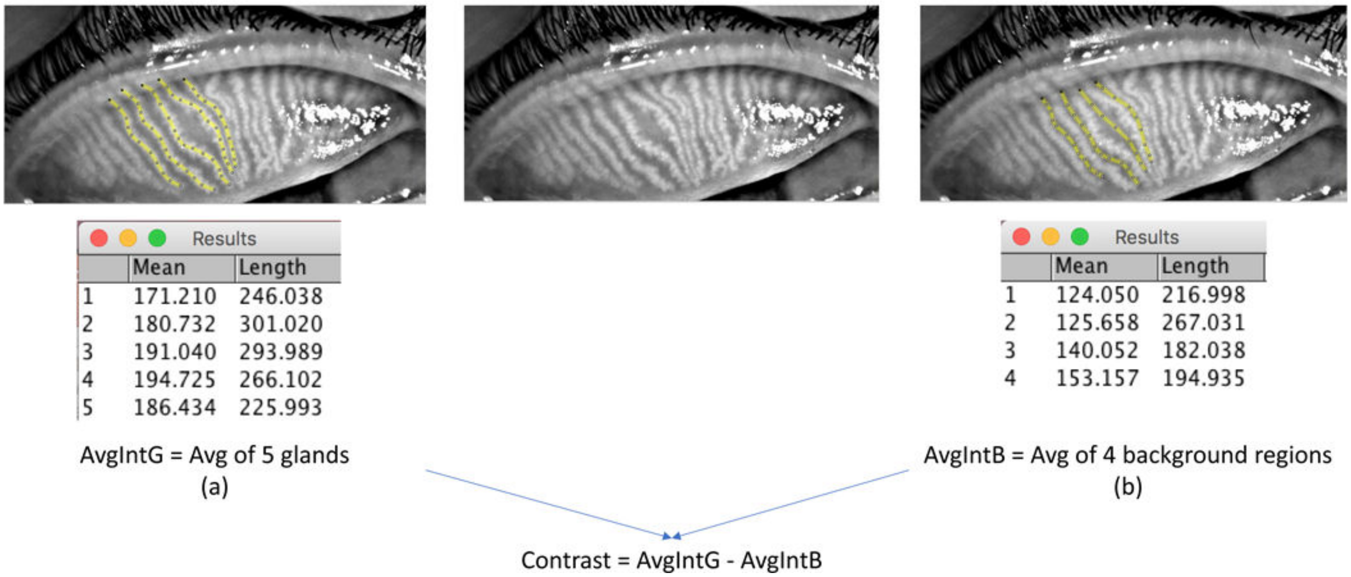

Figure 1.

Contrast is the difference in mean pixel intensity of (a) central 5 glands (AvglntG) (yellow lines represent glands; arrows point to leftmost measured gland) and (b) background intensity between glands (AvglntB) (yellow lines represent background space; arrows point to space between the two leftmost glands).

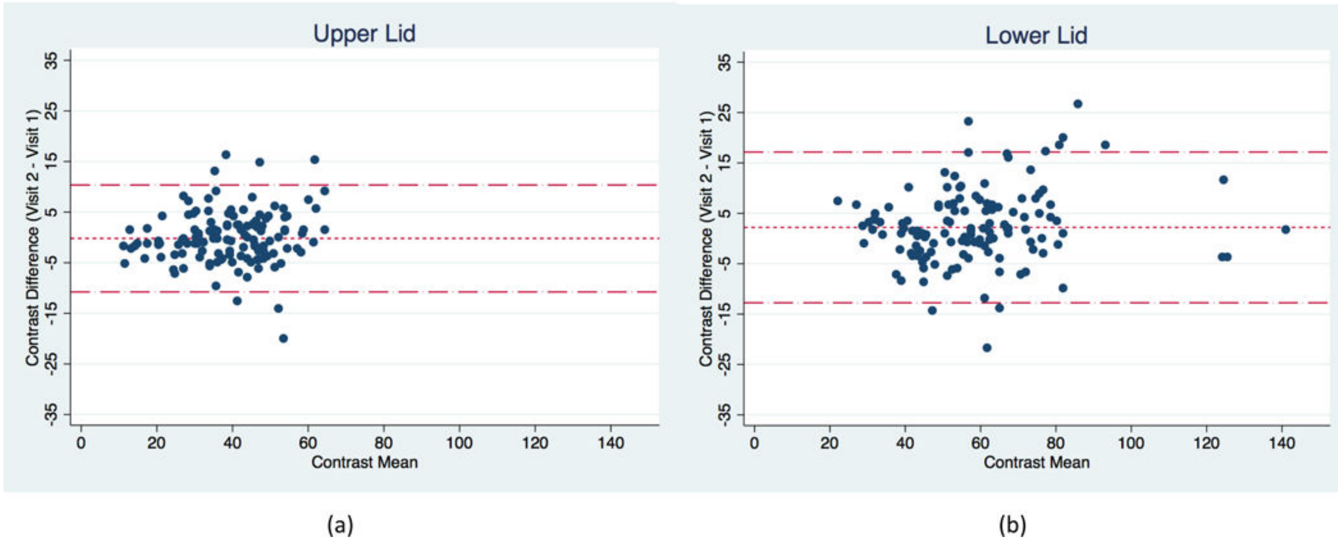

Figure 2.

Contrast Differences vs. Means plots comparing Visit 1 to Visit 2 for (a) upper lid and (b) lower lid.

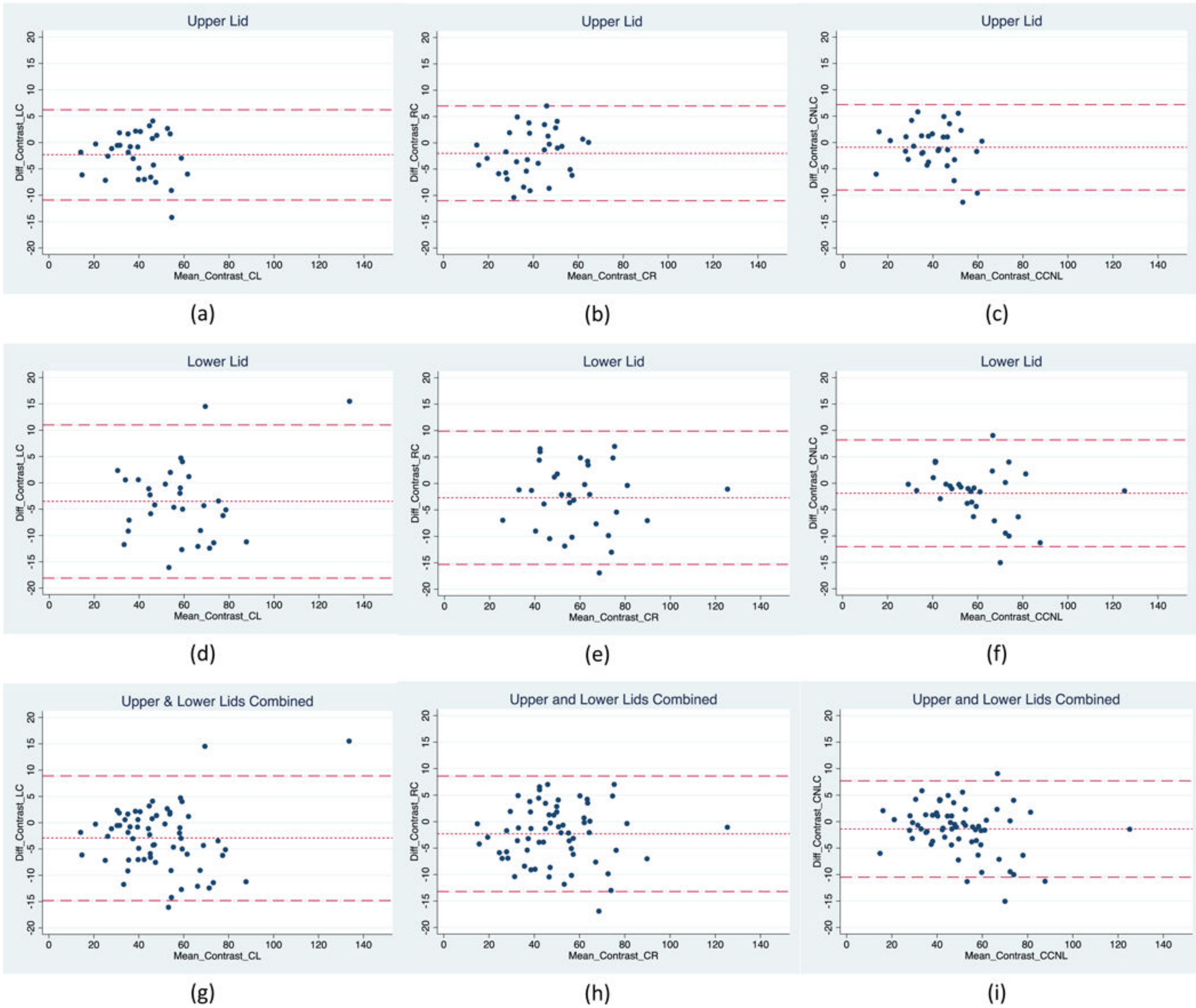

Figure 3.

Contrast Differences vs. 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).

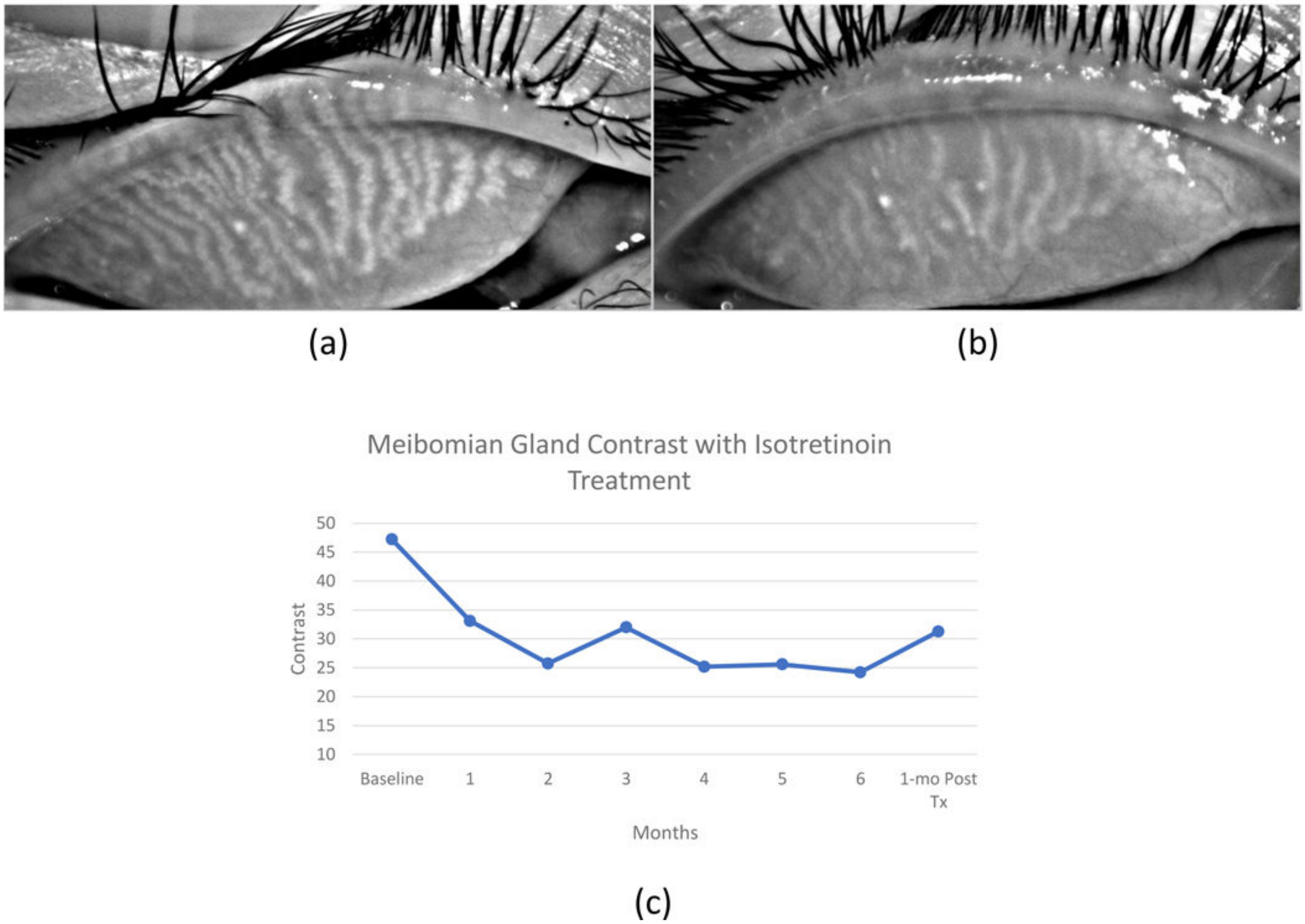

Figure 4.

Isotretinoin patient upper lid meibography images at (a) baseline and (b) after 6 months of treatment, and (c) contrast changes during treatment.

Table 1.

Mean contrast for each test condition at each visit.

	Visit 1	Visit 2
Face-Centered 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 Lights Off		
Upper Lid	39.0 ± 11.0	40.7 ± 12.9
Lower Lid	57.7 ± 18.5	60.0 ± 18.3

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 – 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

Table 3.

Limits of agreement (LOA; matched by visit) for upper lids, lower lids, and all lids.

	Left vs. Center Lights On		Right vs. Center Lights On		Center Lights Off vs. Center 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, −8.3	3.6, 8.8	−13.8, −8.1	4.2, 9.8	−11.6, −6.5	4.7, 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, −13.5	6.5, 15.5	−19.2, −11.3	6.0, 13.9	−15.1, −8.8	5.0, 11.3
Combined						
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, −12.2	6.4, 11.5	−15.5, −10.8	6.2, 10.9	−12.5, −8.6	5.8, 9.7