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Progress in the methods of image analysis of Meibomian glands

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ABSTRACT

Meibography techniques allow one to visualize the silhouette of the morphological structure of the Meibomian gland through illumination of the everted eyelids. The development of non-contact infrared meibography techniques enables meibography to be used routinely during ophthalmological examination. Recently, significant research has been carried out in the field of Meibomian gland image analysis. The subjective and objective methods of the analysis provided a set of descriptive morphological features of the glands. The studies focused on finding a method of Meibomian gland analysis that is objective, quantitative, and less variable and time-consuming for specialists. Moreover, the studies have shown that the evaluation of both the morphology and function of the Meibomian glands provides important clinical information pertaining to Meibomian gland dysfunction (MGD). Meibography alone is not sufficient for the proper diagnosis of MGD but provides a lot of important information that is essential to both improve the treatment protocol and facilitate the diagnosis. This manuscript presents a review of the proposed subjective and objective methods of Meibomian glands image analysis. Moreover, the clinical application of those methods and their relationship with other examinations and symptoms of dry eye disease will be presented. The potential research areas to explore will be identified.

KEY WORDS: Meibomian gland dysfunction, meibography, Meibomian gland image classification, image analysis.

INTRODUCTION

Meibomian gland dysfunction can be characterized as terminal duct obstruction and/or qualitative/quantitative changes in glandular secretion. Meibomian gland dysfunction is caused primarily by terminal duct obstruction as a result of the hyperkeratinization process of the ductal epithelium and increased meibum viscosity. This process leads to stasis of the meibum inside the glands and then dilation and distortion of the ductal system and finally loss of tissue [1-7].

Studies have shown that Meibomian gland dysfunction is a major cause of symptomatic dry eye disease characterized by a loss of homeostasis of the tear film. It may cause ocular surface inflammation, damage and neurosensory abnormalities [1-8].

Meibography techniques allow one to visualize the silhouette of the morphological structure of the Meibomian gland through the illumination of the everted eyelids [9-12]. The development of non-contact meibography by Arita *et al.* (2008) [13] is the beginning of a new era of meibography. Nowadays, there are various multifunctional ophthalmic instruments on the market with a built-in infrared system for meibography. All these factors enable meibography to be used routinely during an ophthalmological examination [13-17].

To classify the obtained meibography images different scales have been proposed. However, there is currently no widely accepted algorithm for detection of those image features that are clinically useful. The technique of image analysis in meibography can help clinicians interpret the degree of gland loss or meibography changes in patients suffering from Meibomian gland dysfunction or ocular surface diseases associated with Meibomian gland dysfunction.

This article presents a short review of the subjective and objective methods used for Meibomian gland image analysis

SUBJECTIVE METHODS OF MEIBOMIAN GLAND ANALYSIS

Most early methods of Meibomian gland image classification were based on the estimation of the dropout area. A grading scale consisting of subjective decisions based on the human experts' experience was proposed [12, 13].

Meibomian glands have been classified as complete or partial [18]. In general, complete Meibomian glands are

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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 [18]. The proposed gestalt grading scale considers the percentage of the image area containing partial Meibomian glands. The following steps were introduced: Grade 1 (no partial glands), Grade 2 (less than 25% of the image contains partial Meibomian glands), Grade 3 (between 25% and 75% of the image contains partial Meibomian glands), and Grade 4 (more than 75% of the image contains partial Meibomian glands) [18].

The second method, introduced by Nichols *et al.* (2008), was based on simply counting the number of complete Meibomian glands, with no credit given for partial glands [18]. The results regarding the within- and between-reader reliability showed a reasonable repeatability score, with the better results reached for the gestalt grading scale. However, there is a significant difference between the within-reader and between-reader reliability. The measurement error associated with the between-reader reliability of meibography images was found to be higher compared with the within-reader reliability [18]. This finding suggests that a reliable analysis of the Meibomian gland images is needed, which would be especially valuable in a clinical trial or epidemiological study in which many readers conduct image assessment. It is worth noting that it was the first evaluation of the reliability of the grading scales for meibography images [18].

The study of Arita *et al.* (2008, 2010) graded partial or complete Meibomian gland loss using the following meibography score for each eyelid: Grade 0 (no loss of Meibomian glands), Grade 1 (loss of less than one-third of the total area of Meibomian glands), Grade 2 (loss of between one-third and two-thirds of the total area), and Grade 3 (loss of over two-thirds of the total area) [19-20]. Then, the meiboscore for the upper and lower eyelids was summed to obtain a score for each eye [19-20]. This method is fully subjective, based on the clinicians' experience; there is no information available according to the within- and between-reader repeatability.

A similar four-grade percentage scale of the Meibomian gland loss in the upper eyelid was introduced by Pflugfelder *et al.* (1998), where meiboscore 0 means no gland dropout, meiboscore 1 means 33% gland dropout, meiboscore 2 means 34-66% gland loss and meiboscore 3 more than 66% means gland dropout [21]. Then, in order to emphasize grading in meibography and to sensitize to minor changes in gland morphology, the 5-grade percentage scale of dropout area was presented [22, 23]. The grading scale describes the meiboscore as follows: meiboscore 1 – area of loss 0%, meiboscore 2 – area of loss < 25%, meiboscore 3 – area of loss 25-50%, meiboscore 4 – area of loss 51-75% and meiboscore 5 – area of loss > 75%. Interestingly, the study showed better inter- and intra-observer agreement for the newly proposed 5-grade scale. Moreover, the five-grade scale gave more consistent increments and facilitated the conversion of the percent gradation to the linear increments, making it more comparable with other subjective grading scales and/or computerized analyses of Meibomian gland loss. Furthermore, smaller increments may enhance the detection of the cut-off values and changes in Meibomian gland morphology [22-23].

Other studies have introduced an application of the semi-computerized method of assessment. The area of the gland loss was measured using ImageJ software (Wayne Rasband, National Institutes of Health, Bethesda, MD) and then the relation of the gland loss to the total lid area was calculated as a Meibomian gland loss factor. It was assumed that the intra- and inter-observer agreement was significantly better using the objective assessment than the subjective grading scales [22]. Similar results were obtained in the study of Srinivasan *et al.* (2012) [24]. Applying ImageJ software provided the first step in the development of the computer assisted methods in meibography. The study concluded that there was a need for a more standardized, automated and less time-consuming method to improve repeatability and precision of the evaluation [22-24].

It was realized that the gland number and the dropout area are not the only features that can be used as Meibomian gland descriptions. Much research has been devoted to quantification of the morphological changes within gland structure. The study of Arita *et al.* (2017) reported the distortion grading, where distortion is characterized by an altered morphology more than 45 degrees in at least 1 Meibomian gland in either the upper or the lower eyelid. The distortion was graded 1 if it was present, and 0 if it was absent. The idea of an all-or-nothing score does not provide the magnitude of the distortion phenomenon. There is only available information about the magnitude of the angle of the distortion for each gland, which does not seem to provide valuable data for an objective classification schema [25].

The study of Pult *et al.* (2012) [26] proposed the use of ImageJ software for Meibomian gland morphology description, namely Meibomian gland thickness and Meibomian gland bending. In the performed analysis, it was assumed that the analysis of one of the worst glands can be considered as a representation of the general stage of Meibomian gland dysfunction [26].

A similar assumption was used in the study of Ban *et al.* (2012) [27]. The Meibomian gland duct length in the upper and lower eyelids was calculated as the average value of the length of the five selected central Meibomian gland ducts.

The other method of Meibomian gland morphology assessment is based on the observation that the healthy Meibomian glands form a parallel stripe pattern throughout the length of the tarsal plate in the eyelids. The presence ("yes") or absence ("no") of tortuosity or non-parallel nature of the glands was noted in the study of Srinivasan *et al.* (2012) [24].

The method of Meibomian gland distortion evaluation presented in the study of Xiao *et al.* (2019) is based on counting the distorted glands, i.e., glands with torsion > 45°. The proposed method is semi-automated and it introduced a cut-off value for Meibomian gland evaluation. The author concluded that the presence of the six distorted

glands was sensitive and specific for the diagnosis of Meibomian gland dysfunction [28].

Meibomian gland morphometric features in children were examined in the study of Zhao *et al.* (2018). The study introduced a 6-grade scale for distortion evaluation, providing valuable information regarding the magnitude of the distortion. There is a lack of information about the usefulness of the proposed scale among the older population [29].

To date, most of the published literature has used subjective scales for meibography image assessment, but recent studies have shown that the use of objective and automated grading may be a more advantageous and suitable method. Developing an objective method of image analysis is challenging but may be the only way to obtain a reliable gland morphology interpretation. Table I presents a summary of the subjective methods of Meibomian gland image analysis.

OBJECTIVE METHODS OF MEIBOMIAN GLAND ANALYSIS

Recently, studies have used image analysis software, namely ImageJ, for semi-computerized analysis and classification of Meibomian gland images [22-27, 30]. Although the user is still involved in identifying the region of interest, better intra-

Table I. Summary of the subjective methods of the Meibomian gland images analysis

Extracted features	Author	Method	Pros	Cons
Complete or partial MGs	Nichols *et al.* (2008)	Counting the number of the complete MGs	Reached reasonable within-reader agreement score	No grading system Low between-reader agreement score Considering only the upper eyelid
	Nichols *et al.* (2008)	The percentage of the image area containing partial MGs	4 Grade scale Reached reasonable within-reader agreement score	Low between-reader agreement score Considering only the upper eyelid
MGs loss	Pflugfelder *et al.* (1998)	The percentage of the image area of the MGs loss	4 Grade scale	Subjective evaluation Considering only the upper eyelid
	Arita *et al.* (2008, 2010)	The part of the area of the MGs loss	4 Grade scale Considering the upper and the lower eyelid	Subjective evaluation
	Pult *et al*. (2013)	The percentage of the image area of the MGs loss	5 Grade scale High within- and between-reader agreement score	Subjective evaluation Considering only the upper eyelid
	Pult *et al.* (2013)	The percentage of the image area of the MGs loss	4 Grade scale Semi-computerized method High within- and between-reader agreement score	Considering only the upper eyelid
	Arita *et al.* (2017)	Observation of the altered morphology more than 45 degrees in at least 1 Meibomian gland	Yes or no scale (0-1 scale) Considering the upper and the lower eyelid	All or nothing scale No information regarding the magnitude of the distortion Subjective evaluation
MGs distortion	Arita *et al.* (2012)	Counting the MGs with altered morphology (with torsion > 45°)	3 Grade scale	Subjective evaluation
	Pult *et al*. (2012)	Measurements of the MGs bending	Semi-computerized method	Analysis of only one of the worst glands as a representation of the overall condition of the MGs No information regarding the magnitude of the distortion
	Xiao *et al*. (2019)	Counting the number of the distorted glands (with torsion > 45°)	Proposed cut-off value of the six distorted glands for MGD diagnosis Semi-computerized method	Considering only the upper eyelid subjective evaluation
	Zhao *et al.* (2018)	Considering the amount of the distortion and the area of the distorted MGs	6 Grade scale Information regarding the magnitude of the distortion	Considering only the upper eyelid Examined in the group of children

and inter-observer agreement have been achieved [22, 31-32]. Hence, a need has been expressed for a method of meibography image assessment that is fast, less laborious and less dependent on the experience of the examiners [22, 31-33].

Nonetheless, it is important to mention how challenging the Meibomian gland images could be for the image processing tools. The difficulties consist of low contrast, out-of-focus and non-uniformly illuminated images. Furthermore, artifacts appear in the obtained images, such as specular reflection from the tear film or eyelashes interfering with the detection of the glands. Another feature of the meibographic images is that pixel intensity changes gradually between the background and glands, making gland segmentation more difficult [34-35].

The study of Koh *et al.* (2012) provided the first step in the development of an objective method of Meibomian gland analysis by introducing an algorithm detecting the lines along the center and between the glands (called the gland and inter-gland lines) and the width of the glands [34]. The extraction of these features was based on finding the location of maximum and minimum pixel intensity of the pre-processing image. The gland centers and inter-gland points are located at the local maxima and minima of this profile, where the gradient of the pixel intensity vanishes. For the classification, the average arc length of all the lines (gland and inter-gland lines) was used. Furthermore, the detection of the width of the gland was performed using the scale-invariant feature transform, which allows the detection and estimation of the local changes in the image. The Shannon entropy (measure of the uniformity in distribution) method was used to capture the difference in the local distributions between healthy and unhealthy Meibomian glands and thereby two descriptive parameters of gland i.e., average entropy and average scale for classification, were reached. Then, a support-vector machine was used to combine the morphological features and to classify the gland images into two categories – healthy and unhealthy. Satisfactory results in terms of classification efficiency were achieved, namely for the testing data, a specificity of 96% and sensitivity of 98%. However, the current algorithm failed to produce satisfactory results when an intermediate group was proposed. However, this method was not fully automated as the area of the eyelid interest was selected manually. The study considered only the upper eyelid.

The main observation was that the intermediate group tends to overlap with the healthy and unhealthy class. Therefore, a larger set of descriptive gland morphological features should be assessed to improve the classification of the intermediate group. One of the limitations of this method is the necessity to use image pre-processing tools. On the other hand, the proposed method does not require the use of image editing software to manually indicate the area of interest i.e. the regions with and without Meibomian glands, which undoubtedly makes this procedure less subjective and more reliable. The study highlights the need for an algorithm to automatically locate the area of interest. So far, users have drawn manually the region of the eyelid and have decided about the location of the regions with and without glands. The author suggests several possible solutions to this problem. The automated analysis may be achieved by simple changes in the imaging protocol, namely changing the magnification to obtain an image with a visible upper eyelid margin and the edge of the upper tarsal plate, then the lid margin and the edge can be used as the references lines to define the gland region [34]. It is worth adding that although admittedly the proposed method provides new parameters of the Meibomian gland morphology (central length of the detected Meibomian glands and spaces between neighboring glands), based on the previous studies, these morphological features are not necessarily associated with Meibomian gland dysfunction [19].

Recently, Celik *et al.* (2013) [36] proposed a new fully automated approach. In this study the meibography images were classified into three classes. The method is based on Gabor wavelet filtering, which is utilized as local filtering due to its parameterization in shape, local spatial support, and orientation. The lengths and widths of the gland and inter-gland structures are estimated using the mid-lines of the structures. The detected mid-lines of the gland and inter-gland regions are used to extract four descriptive parameters for a given image. Next, these features were combined using support-vector machines for classification. The automatic classification was compared with the ground truth classification made by clinicians. The classification performance reached 100% accuracy for classifying images into two (healthy and unhealthy) classes and 88% accuracy for classifying into three (healthy, intermediate and unhealthy) categories. The small number of samples should be taken into consideration; namely the classification was performed only on a set of 65 Meibomian gland images. The author pointed out that future work should focus on the segmentation of the Meibomian gland images into three regions representing the area of the gland, the inter-gland region (i.e., healthy tissue with no glands), and areas of tissue loss as a further improvement of the classification performance [36].

A different automated morphological analysis of the Meibomian gland structures was proposed by Llorens-Quintana *et al.* (2019) [37]. Additionally, using the proposed approach, the impact of Meibomian gland morphology changes on gland function and ocular surface was investigated [35]. The proposed method provided the area of interest, which is the area of the tarsal plate containing Meibomian glands. As the next step, the individual Meibomian glands were isolated and the set of morphological features, i.e., dropout area, gland length, gland width, number of glands, and gland irregularity, was extracted. Interestingly, the length and width of the glands were estimated by fitting an ellipse having the same normalized central moment for the given gland. An ellipse represents the data distribution fitted to a single gland, considering it as a two-dimensional object. The length and width of the gland are described as an approximation to the length of the major and minor axis of the fitted ellipse, respectively. Afterwards, the mean value of both parameters for every image for all the exposed glands of the tarsal conjunctiva was computed [35, 37]. Additionally, the study introduced meas-

urements of gland irregularity, i.e., shape dissimilarity of each gland from the standardized regular gland. The standardized shape of the gland was determined by normalizing the edge coordinates of regular glands. Gland irregularity was defined as the differential area between the standard gland and the examined gland after the superposition of the shape of each detected gland onto the shape of the standard gland and was presented as the percentage area of the total regular gland, which can be defined as the amount of the gland that is outside of the regular shape. Then, the mean value for the whole set of glands was calculated and used for further procedures. As reported earlier, also in this study, in a few cases, the algorithm failed to properly select the tarsal conjunctiva area, which again highlights the importance of the image acquisition and pre-processing [37]. Despite this fact, the method was highly successful in isolating the glands and performing quantitative analysis of the glands. This is the first time that an automated method has described the irregularity of the glands. There is a lack of information about a classification based on the described parameters.

One of the first studies presenting software that automatically calculates the ratio of Meibomian gland loss area to total area was presented by Arita *et al.* (2014) [19]. A newly developed approach determined the lid borders, and then a set of filters was used to automatically discriminate the Meibomian gland area. The preprocessing analysis consisted of applying a Wallis filter to enhance the areas of the low contrast, then a Gaussian filter to reduce the image noise. Using the set of sophisticated methods, the area of the everted eyelid was automatically detected. Then, the Meibomian gland area was extracted after using correction filters. The ratio of the Meibomian gland area to the total examined area for each upper and lower eyelid was calculated and compared with the subjective evaluation. From the entire set of the obtained images, only two of them required manual correction, one because the large area of the strong reflection wrongly classified as a Meibomian gland area, and the other due to extensive loss of the Meibomian glands. However, based on the obtained repeatability score, it can be concluded that the method is highly reliable; namely, the intra-examiner coefficients of variation for the objective analysis of upper/lower Meibomian gland area in the control group and in group of the patients with MGD were 0.59%/0.40% and 0.47/0.44%, respectively. Some limitations of the study could not be overcome. First, images with specular reflections or images presenting excessive Meibomian gland loss required manual revision and correction. Secondly, there is a need to clarify the variation of findings in the terminal part of the glands; namely, in the study a decrease in the image quality of the terminal part of the glands with age and lipid profile content was observed. Therefore, this finding requires further investigations [19]. In order to better understand the Meibomian gland morphology changes in the course of Meibomian gland dysfunction, a comparison of the distal, mid and proximal zones of the gland regions should be conducted. Moreover, the study of Andrews *et al.* (2020) [38] showed significant differences in meibography grading between regional zones (nasal, central, temporal) and global grades. This observation also should be considered in further research.

The study of Ciezar *et al.* (2020) [39] focused on the extraction of the morphometric features of the Meibomian glands and improvements in the classification performance of the Meibomian gland images. The proposed approach is not based on a description at the level of individual glands, but on the global analysis of the whole eyelid area using a 2D Fourier transform of the whole set of glands. As a result, two objective grading parameters, namely gland mean frequency and anisotropy in gland periodicity, were obtained. Recorded images were subjectively graded by the experts and grouped into three categories: healthy, intermediate and unhealthy. The results suggest that combining more parameters in an automatic decision process can be a way of significantly improving the performance of any future classification scheme. Some limitations of this method result from its global character. During the full eyelid analysis an image containing a structure in which glands, although forming a periodic structure, are inclined at various different angles may be incorrectly characterized by a high value of the anisotropy parameter and so incorrectly classified. To solve this problem, a similar analysis but performed on the local scale (analyzing small sections of the whole image) should be considered [39].

Nowadays, the deep learning approach, a particular form of artificial intelligence, is gaining more attention in medical imaging [40-41]. Deep learning is an artificial intelligence function that imitates the workings of the human brain in processing data and creating patterns for use in decision-making. These advanced methods can predict features directly from a large dataset of labeled images, without explicitly specifying rules or features [42-43]. A four-stage meiboscore was used in this study and it was found that the numeric percent atrophy is a better rating method than the four-stage meiboscore, especially for samples where the percent atrophy is near the grading transition limits [44]. The evaluation performed by human experts was compared with those performed by the deep learning approach. Satisfactory results were obtained; namely, the accuracy of the determination of the eyelid area and atrophy area was 97.6% and 95.4%, respectively [44]. The results show that deep learning systems combined with experience-based knowledge of the clinicians may be the future of image-based medical diagnosis [42-44]. Further work should focus on the ability to predict the individual gland morphology by the deep learning approach.

It is worth noting that until now there are only a few studies using a deep learning approach for Meibomian gland dysfunction diagnosis, but the results so far are very promising. Success in automatic parameterization of the images based on dropout area gives hope that these methods will be equally successful in describing the Meibomian gland using different morphometric features. Therefore, further research should be aimed at searching for descriptive parameters of the Meibomian gland and linking these features with ocular surface condition and tear film parameters. Such data would make it

possible, in the near future, to train the neural network to find more subtle changes in the meibography images and diagnose Meibomian gland dysfunction at an early stage. Table II presents a summary of objective methods of Meibomian gland images analysis.

CLINICAL PRACTICE

Some studies have evaluated subjective Meibomian gland image analysis with ocular surface parameters. However, most of them concentrated on the dropout area, excluding the morphological features. A significant correlation of dropout area with ocular symptoms, lid abnormalities, fluorescein breakup time, and gland expressibility has been reported [23, 45-51].

Several studies have used a semi-automated Meibomian gland image analysis and found a relationship between dropout area and noninvasive breakup time and Ocular Surface Disease Index score [23, 26]. In only one available study using the fully automated Meibomian gland analysis algorithm, a positive significant correlation between dropout area and bulbar conjunctival hyperemia and conjunctival staining was found [37].

It should certainly be noted that the obtained results may vary among the studies. Several factors should be mentioned, i.e., the method of assessment (the level of subjectivity in the image analysis) and the set of obtained meibography images (a large mixed group of healthy and unhealthy images is desirable) [35-36].

Even though the Meibomian gland morphology may play a crucial role in Meibomian gland disease, there is a limited number of studies investigating the influence of Meibomian gland morphology on Meibomian gland function, tear film characteristics, and ocular surface health. The study of Ban *et al*. (2013) [27] showed the correlations between gland length and Meibomian gland expressivity. A significant relation was found between the gland length and Ocular Surface Disease Index and non-invasive breakup time score in the study of Pult *et al*. (2012) [23-26] using the semi-automated analysis method.

Table II. Summary of the objective methods of the Meibomian gland images analysis

Extracted features	Author	Method	Pros	Cons
MGs length and width	Koh *et al*. (2012)	Scale Invariant Feature Transform, Shannon Entropy	Fully automated method Proposed MGs image classification into 2 classes	Obtained morphological features not necessarily associated with MGD Considering only the upper eyelid
MGs length and width and inter-gland structures	Celik *et al*. (2013)	Gabor wavelets filtering	Automated features extraction Proposed MGs image classification into 3 classes	Simplification of the area of interest as an ellipse Considering only the upper eyelid Small number of samples
MGs length and width, number of glands, MGs irregularity, dropout area	Llorens-Quintana *et al*. (2019)	Set of sophisticated methods, including estimation the differences between standardized MGs and examined MGs	Fully automated method Automated extraction of the area of interest New limits for drop-out grading Finding correlations of the morphometric features with ocular surface parameters	Lack of the Meibomian gland image classification based on all extracted features Difficulties by extracting the area of interest, manual correction needed Considering only the upper eyelid
Drop out area	Arita *et al.* (2014)	Set of sophisticated methods, including discrimination analysis method, erosion image processing, edge detection	Fully automated method Automated extraction of the area of interest Considering the upper and the lower eyelid	Manually correction needed by an excessive Meibomian gland loss Difficulties by finding the border of the area of gland in terminal part of the glands
Drop out area	Wang *et al.* (2019, 2020)	Deep learning approach	Fully automated method Automated extraction of the area of interest Numeric percent atrophy, no grading scale	Considering only the upper eyelid No information about the ability to predict the individual gland morphology
MGs mean frequency and anisotropy in gland periodicity	Ciezar *et al*. (2020)	2D Fourier transform	Proposed Meibomian gland image classification into 3 classes Automated features extraction	Manually defined area of interest Global analysis of the whole set of the MGs not of the individual MGs morphology Considering only the upper eyelid

It has been suggested that the gland irregularity may be related to the quality and quantity of the expressed meibum [24, 52]. However, there is still a need for further work in this area due to there being only one available study investigating the objective value of gland irregularity with gland function and ocular surface condition [37].

In the study of Xiao *et al*. (2019) both dropout area and gland distortion were correlated with dry eye severity level, meibum expressibility, and meibum quality. The authors suggested the necessity of Meibomian gland morphology analysis in MGD development. Moreover, the evaluation of Meibomian gland loss and distortion provide valuable complementary clinical parameters to assess MGD status [28].

A significant correlation was found between Meibomian gland tortuosity and the lid margin score, meiboscore, meibum expressibility score, and breakup time [52].

The presented studies concluded that gland distortion may be an early indicator of MGD and associated with progressive loss of the Meibomian glands in the advanced stage of MGD [28]. Similar findings in the study of Lin *et al.* (2020) suggest that Meibomian gland tortuosity may play an important role in the diagnosis of MGD severity, especially in the early stage of the disease [52-53].

Interestingly, in the study of Pistilli *et al*. (2020) most of the proposed Meibomian gland morphologic features were not associated with the severity of the dry eye disease symptoms or signs, but correlations between tortuous glands and higher tear breakup time and longer Schirmer test length were noted [54].

In conclusion, according to the current knowledge, meibography alone does not appear to be sufficient for Meibomian gland dysfunction diagnosis, but instead should be interpreted in the context of the other clinical parameters [49-52, 55]. Nonetheless, it remains unclear which of the morphometric features of the glands have a significant impact on the gland function and ocular surface condition [36-37, 55].

CONCLUSIONS

According to what has been found in the existing research reports, the objective analysis of meibographic images has already proved to be a useful technique improving the treatment protocol for Meibomian gland dysfunction and ocular surface diseases associated with Meibomian gland dysfunction [49]. Therefore, it is crucial to further develop an objective and automatic assessment system of the Meibomian gland images that would provide the dropout area and the morphologic parameters. A quantitative, less variable and time-consuming method for Meibomian gland evaluation could bring new insights for understanding and diagnosis of Meibomian gland dysfunction.

It is necessary to test the reproducibility of the presented methods. To our knowledge, there is no study comparing the results obtained using a different set of images. It is crucial to establish one general, not instrument-specific method. Properties such as obtained image size, different image resolution and other potential differences, such as level of illumination, must be considered. The proposed method should be easily adapted to any possible changes caused by the image acquisition method. A possible future improvement is the idea to create an extensive open-access database of Meibomian gland images that will serve as a test source for future automatic analysis algorithms. In order to directly compare different analytic approaches, it would be convenient to test them with exactly the same set of gland images. This would allow a truly objective comparison between performance of the different algorithms and hopefully set the standard of the most proper Meibomian image analysis protocols.

Future work should be devoted to finding new objective measures of imaged gland structures and trying to connect them with the gland physiological condition, as well as the ocular surface condition and tear film parameters. Having knowledge of these correlations, the sophisticated machine learning algorithms can use such data for automatic detection of the relevant gland features and then show potential connections with other physiological parameters. It would be extremely helpful and could greatly assist in diagnostic decision making.

Although there is strong evidence that the Meibomian image contains a lot of important information, it will never be the only source of the information. Therefore, the diagnosis of Meibomian gland dysfunction will never be based solely on the imaging technique. However, considering the short time and low cost of data acquisition, informative capacity and the patient's comfort during this procedure, Meibomian gland imaging enhanced by a proper image analysis algorithm has the potential to become the primary diagnostic method.

DISCLOSURE

The authors declare no conflict of interest.

References

1. Nichols KK, Foulks GN, Bron AJ, et al. The international workshop on Meibomian gland dysfunction: executive summary. Invest Ophthalmol Vis Sci 2011; 52: 1922-1929.
2. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. Invest Ophthalmol Vis Sci 2011; 52: 1930-1937.
3. Millar TJ, Schuett BS. The real reason for having a Meibomian lipid layer covering the outer surface of the tear film – a review. Exp Eye Res 2015; 137: 125-138.
4. Knop E, Knop N, Millar T, et al. 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: 1938-1978.

5. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. Invest Ophthalmol Vis Sci 2011; 52: 2006-2049.
6. Robin JB, Nobe JR, Suarez E, Jester JV, Smith RE. Meibomian gland evaluation in patients with extended wear soft contact lens deposits. CLAO J 1986; 12: 95-98.
7. Bron AJ, de Paiva CS, Chauhan SK, et al. TFOS DEWS II pathophysiology report. Ocul Surf 2017; 15: 438-510.
8. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II Definition and Classification Report. Ocul Surf 2017; 15: 276-283.
9. Robin JB, Jester JV, Nobe J, Nicolaides N, Smith RE. In vivo transillumination biomicroscopy and photography of meibomian gland dysfunction. A clinical study. Ophthalmology 1985; 92: 1423-1426.
10. Mathers WD, Daley T, Verdick R. Video imaging of the meibomian gland. Arch Ophthalmol 1994; 112: 448-449.
11. Yokoi N, Komuro A, Yamada H, et al. A newly developed video-meibography system featuring a newly designed probe. Jpn J Ophthalmol 2007; 51: 53-56.
12. Pult H, Nichols JJ. A review of meibography. Optom Vis Sci 2012; 89: E760-E769.
13. 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: 911-915.
14. 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-E315.
15. Srinivasan S, Menzies K, Sorbara L, Jones L. Infrared imaging of meibomian gland structure using a novel keratograph. Optom Vis Sci 2012; 89: 788-794.
16. Ngo W, Srinivasan S, Jones L. Historical overview of imaging the Meibomian glands. Journal of Optometry 2013; 6: 1-8.
17. Yoo YS, Na KS, Kim DY, et al. Morphological evaluation for diagnosis of dry eye related to meibomian gland dysfunction. Exp Eye Res 2017; 163: 72-77.
18. Nichols JJ, Berntsen DA, Mitchell GL, Nichols KK. An assessment of grading scales for meibography images. Cornea 2005; 24: 382-388.
19. Arita R, Suehiro J, Haraguchi T, et al. Objective image analysis of the meibomian gland area. Br J Ophthalmol 2014; 98: 746-755.
20. Arita R, Itoh K, Maeda S, et al. Meibomian gland duct distortion in patients with perennial allergic conjunctivitis. Cornea 2010; 29: 858-860.
21. Pflugfelder SC, Tseng SC, Sanabria O, et al. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. Cornea 1998; 17: 38-56.
22. Pult H, Riede-Pult B. Comparison of subjective grading and objective assessment in meibography. Cont Lens Anterior Eye 2013; 36: 22-27.
23. Pult H, Riede-Pult BH. Non-contact meibography: keep it simple but effective. Cont Lens Anterior Eye 2012; 35: 77-80.
24. Srinivasan S, Menzies K, Sorbara L, Jones L. Infrared imaging of meibomian gland structure using a novel keratograph. Optom Vis Sci 2012; 89: 788-794.
25. Arita R, Fukuoka S, Morishige N. Meibomian Gland Dysfunction and Contact Lens Discomfort. Eye Contact Lens 2017; 43: 17-22.
26. 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-E315.
27. Ban Y, Shimazaki-Den S, Tsubota K, Shimazaki J. Morphological evaluation of meibomian glands using noncontact infrared meibography. Ocul Surf 2013; 11: 47-53.
28. Xiao J, Adil MY, Olafsson J, et al. Diagnostic Test Efficacy of Meibomian Gland Morphology and Function. Sci Rep 2019; 9: 17345.
29. Zhao Y, Chen S, Wang S, et al. The significance of meibomian gland changes in asymptomatic children. Ocul Surf 2018; 16: 301-305.
30. Menzies KL, Srinivasan S, Prokopich CL, Jones L. Infrared imaging of meibomian glands and evaluation of the lipid layer in Sjögren's syndrome patients and nondry eye controls. Invest Ophthalmol Vis Sci 2015; 56: 836-841.
31. Nichols JJ, Berntsen DA, Mitchell GL, Nichols KK. An assessment of grading scales for meibography images. Cornea 2005; 24: 382-388.
32. Wolffsohn JS. Incremental nature of anterior eye grading scales determined by objective image analysis. Br J Ophthalmol 2004; 88: 1434-1438.
33. O'Dell L., Halleran C, Schwartz S, et al. An assessment of subjective meibography image grading between observers and the impact formal gland interpretation training on inter-observer agreement of grading scores. Invest Ophthalmol Vis Sci 2020; 61: 486-486.
34. Koh YW, Celik T, Lee HK, et al. Detection of meibomian glands and classification of meibography images. J Biomed Opt 2012; 17: 086008.
35. Llorens-Quintana C, Rico-del-Viejo L, Syga P, et al. A novel automated approach for infrared-based assessment of Meibomian gland morphology. Trans Vision Sci Tech 2019; 8: 17.
36. Celik T, Lee HK, Petznick A, Tong L. Bioimage informatics approach to automated Meibomian gland analysis in infrared images of meibography. J Optometry 2013; 6: 194-204.
37. Llorens-Quintana C, Rico-del-Viejo L, Syga P, et al. Meibomian gland morphology: the influence of structural variations on gland function and ocular surface parameters. Cornea 2019; 38: 1506-1512.
38. Andrews JD, O'Dell L, Halleran C, et al. Evaluation of meibography images should consider regional assessment to Meibomian gland structural characteristics as well as global grading scales. Invest Ophthalmol Visual Sci 2020; 61: 2633-2633.
39. Ciężar K, Pochylski M. 2D fourier transform for global analysis and classification of Meibomian gland images. Ocular Surface 2020; 18: 865-870.
40. Ting DSW, Cheung CY, Lim G, et al. Development and Validation of a Deep Learning System for Diabetic Retinopathy and Related Eye Diseases Using Retinal Images From Multiethnic Populations With Diabetes. JAMA 2017; 318: 2211-2223.
41. Nayak J, Bhat PS, Acharya R, et al. Automated identification of diabetic retinopathy stages using digital fundus images. J Med Syst 2008; 32: 107-115.
42. Wang J, Li S, Yeh TN, et al. Meibomian Gland Morphology and Ghost Prediction with Deep Learning. Invest Ophthalmol Visual Sci 2020; 61: 2634-2634.
43. Wang J, Yeh TN, Chakraborty R, et al. A deep learning approach for Meibomian gland atrophy evaluation in meibography images. Transl Vis Sci Technol 2019; 8: 37.
44. Maruoka S, Tabuchi H, Nagasato D, et al. Deep neural network-based method for detecting obstructive Meibomian gland dysfunction with in vivo laser confocal microscopy. Cornea 2020; 39: 720-725.

45. Arita R, Itoh K, Maeda S, et al. Proposed diagnostic criteria for obstructive Meibomian gland dysfunction. Ophthalmology 2009; 116: 2058-2063.
46. Machalinska A, Zakrzewska A, Adamek B, et al. Comparison of morphological and functional Meibomian gland characteristics between daily contact lens wearers and nonwearers. Cornea 2015; 34: 1098-1104.
47. Eom Y, Lee JS, Kang SY, et al. Correlation between quantitative measurements of tear film lipid layer thickness and Meibomian gland loss in patients with obstructive meibomian gland dysfunction and normal controls. Am J Ophthalmol 2013; 155: 1104-1110.e2.
48. Sevim DG, Gumus K, Unlu M. Reliable, noncontact imaging tool for the evaluation of Meibomian gland function: sirius meibography. Eye Contact Lens 2020; 46 Suppl 2: S135-S140.
49. Arita R, Fukuoka S, Kawashima M. Proposed Algorithm for Management of Meibomian Gland Dysfunction Based on Noninvasive Meibography. J Clin Med 2021; 10: 65.
50. Adil MY, Xiao J, Olafsson J, et al. Meibomian gland morphology is a sensitive early indicator of Meibomian gland dysfunction. Am J Ophthalmol 2019; 200: 16-25.
51. Arita R, Itoh K, Maeda S, et al. Proposed diagnostic criteria for obstructive Meibomian gland dysfunction. Ophthalmology 2009; 116: 2058-2063.
52. Lin X, Fu Y, Li L. A novel quantitative index of Meibomian gland dysfunction, the Meibomian gland tortuosity. Transl Vis Sci Technol 2020; 9: 34.
53. Villani E, Marelli L, Dellavalle A. Latest evidences on Meibomian gland dysfunction diagnosis and management. The Ocular Surface 2020; 18: 871-892.
54. Daniel E, Pistilli M, Ying GS, et al. Association of Meibomian gland morphology with symptoms and signs of dry eye disease in the Dry Eye Assessment and Management (DREAM) study. Ocular Surface 2020; 18: 761-769.
55. Giannaccare G, Vigo L, Pellegrini M, et al. Ocular surface workup with automated noninvasive measurements for the diagnosis of Meibomian gland dysfunction. Cornea 2018; 37: 740-745.