Automated Segmentation of Foveal Avascular Zone in Fundus Fluorescein Angiography

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Purpose. To describe and evaluate the performance of a computerized automated segmentation technique for use in quantification of the foveal avascular zone (FAZ).

Methods. A computerized technique for automated segmentation of the FAZ using images from fundus fluorescein angiography (FFA) was applied to 26 transit-phase images obtained from patients with various grades of diabetic retinopathy. The area containing the FAZ zone was first extracted from the original image and smoothed by a Gaussian kernel (σ = 1.5). An initializing contour was manually placed inside the FAZ of the smoothed image and iteratively moved by the segmentation program toward the FAZ boundary. Five tests with different initializing curves were run on each of 26 images to assess reproducibility. The accuracy of the program was also validated by comparing results obtained by the program with the FAZ boundaries manually delineated by medical retina specialists. Interobserver performance was then evaluated by comparing delineations from two of the experts.

Results. One-way analysis of variance indicated that the disparities between different tests were not statistically significant, signifying excellent reproducibility for the computer program. There was a statistically significant linear correlation between the results obtained by automation and manual delineations by experts.

Conclusions. This automated segmentation program can produce highly reproducible results that are comparable to those made by clinical experts. It has the potential to assist in the detection and management of foveal ischemia and to be integrated into automated grading systems. (*Invest Ophthalmol Vis Sci.* 2010;51:3653–3659) DOI:10.1167/iovs.09-4935

Cumulative damage within the ocular microcirculation in patients with diabetes mellitus results in the specific pathophysiological changes of diabetic retinopathy. In diabetic maculopathy, microangiopathy is evident as both microvascular occlusion and leakage, either from microaneurysms or from increased permeability of capillaries. The disturbance of macular hemodynamics is associated with a decrease in visual acuity. Hence assessment of the macular circulation is a central step in the clinical management of diabetic retinopathy.

The severity of diabetic maculopathy can be partly established by examining the perifoveal circulation.[1–8] The central macula has a foveal avascular zone (FAZ) surrounded by interconnected capillary beds. This vascular network terminates in the central macula and forms a ring at the peripheral edge of the FAZ. Depending on the vascular pattern, the physiological shape of the FAZ is oval or round and has an average diameter of 500 to 600 μm. Diabetes-related damage to the macular circulation induces a number of changes in the vasculature. Enlargement of the FAZ observed on fundus angiography of the diabetic eye may indicate a poor prognostic visual outcome. Figure 1A illustrates a transit-phase image acquired from a patient with nonproliferative diabetic retinopathy (NPDR) in whom the FAZ is observed as a hypofluorescent area in the center of the macula. Figure 1B shows a magnified portion of Figure 1A and demonstrates the irregularity of the FAZ outline. Bresnick et al.[9] reported a significant enlargement of the FAZ in patients with diabetic retinopathy compared with controls and found a strong correlation between the size of the FAZ and the severity of nonperfusion in the posterior retina, indicating that angiographic delineation of the FAZ is highly relevant in the assessment and management of diabetic eye disease.[9]

Until the recent advent of computerized analysis, the diabetic macula has been assessed qualitatively using the guidance set by the Early Treatment Diabetic Retinopathy Study (ETDRS).[10] During this process of manual image grading, the observer produces a descriptive summary that mentions features such as the extent of capillary nonperfusion and the regularity of the FAZ outline. Limitations of this traditional approach include intraobserver and interobserver variability and only approximate measurements of FAZ diameter. Traditionally, the size and shape of the FAZ has been determined manually by trained image graders following the ETDRS protocol for the purpose of clinical research. This process is laborious and time consuming and is compromised by poor image quality and interobserver and intraobserver variability. Accordingly, most published studies describing the FAZ outline have used only high-quality angiograms. Although recent interest has been directed toward the development of computerized programs to improve the assessment of diabetic retinopathy,[11] angiographic evaluation of the FAZ using such automated segmentation techniques remains a poorly explored subject despite its clear clinical applicability.

Few published studies are available that illustrate the potential of computerized techniques in evaluating the diabetic macular circulation.[12–14] Genetic snakes[12] and a Bayesian statistical method[13] showed promising results in automated FAZ segmentation, but no validation work has been undertaken to determine the reliability and reproducibility of the technique. In another recent study, a region-growing–based technique was proposed but was applied only to preselected high-quality fundus fluorescein angiography (FFA) images.[14] Performance

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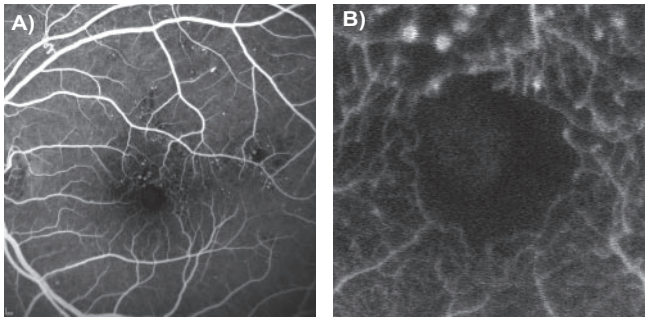

FIGURE 1. (**A**) Illustration of an FFA image. (**B**) A 256 × 256 subimage of (**A**) containing FAZ.

was evaluated by comparison with annotations made by clinicians. However, the validation result was not promising because the FAZ size measured by the program was significantly smaller than that on clinician assessment. This difference arose partly because of limitations of the region-growing algorithm in handling noise and variations in image intensity.

Given the experience from this study and that of others, level-set–based image segmentation has the potential to be a useful technique in a clinical setting.[12–14] The automated segmentation technique described in this article was developed based on a variational level-set method.[15] Level-set techniques have been used widely for the detection of interfaces in various medical imaging applications, including retinal image analysis. Because of their superiority in tackling complex segmentation problems in a mathematically efficient manner, these techniques are being increasingly explored for image analysis in clinical medicine.[16] The step of image segmentation has been a major challenge in the field of image processing. A plethora of techniques (such as graph-cut and classifier-based methods) have been developed to address image segmentation. All these methods have their own merits and limitations. For example, classifier-based techniques such as artificial neural network segmentation require the building of a large database before application of the technique, additionally posing a challenge in the identification of deterministic features of the target objects. The purpose of this study was to evaluate the performance of a new automated segmentation technique based on level-set methods for measuring the size and shape of the FAZ in routine clinical practice. Combining advances in image analysis with a rigorous mathematical framework, we believe that automated angiographic grading of maculopathy can be further refined.

PATIENTS AND METHODS

A total of 26 FFA images (15 left eyes, 11 right eyes) from 26 patients with different severities of diabetic retinopathy were collected in a prospective observational study. The study was performed in adherence to the tenets of the Declaration of Helsinki.

Patient Characteristics

There were 20 male and 6 female patients (mean age, 53 ± 12 years; range, 25–79 years). Retinopathy stage in the study eye was categorized according to the extended Modified Airlie House Classification developed by the ETDRS.[17] One eye had no signs of retinopathy, seven had mild NPDR, seven had moderate NPDR, seven had severe NPDR, and four had proliferative diabetic retinopathy. Twelve eyes also had clinically significant macular edema. Seven eyes had undergone focal macular laser treatment, and five had undergone panretinal photocoagulation treatment before the study. In eyes that previously underwent focal macular laser treatment, the mean distance between the FAZ and the nearest laser spot was 0.39 mm (range, 0.30–0.55 mm).

Fluorescein Angiography

Fluorescein (3 mL 25% dye) angiography was performed after dilatation of the pupil. Digital images were taken using a scanning laser ophthalmoscope (HRA2; Heidelberg Engineering, Heidelberg, Germany) in high-resolution mode. During the transit phase, 30° images of the fovea of the study eye were obtained. The quality of these images was reviewed by a panel of experts consisting of two or more medical retina specialists (JSG, ANS, CC, DMB) at any single review session, and consensus was reached regarding whether quality was sufficient for clinical management. The best available transit phase image of the study eye of each patient was identified, and the images were then exported in BMP format for the purpose of analysis.

Image Analysis

The program was written in commercial software (MatLab R14; The MathWorks Inc., Natick, MA). All the segmentation tasks were run on a computer (configurations: Windows XP Service Pack 2 [Microsoft Corporation, Redmond, WA], Intel Core 2 [Intel Corporation, Mountain View, CA], 2.66 GHz, and 3.25 GB of RAM).

Automated Segmentation Program. We used a variational level-set method initially described by Li et al.[15] As with other level-set approaches, segmentation was achieved by determining the boundary of the object by evolving a contour represented as zero level of a signed function. The signed function here derives from the framework of energy minimization. In particular, the energy under consideration consists of three parts: the first part is a gradient-regularized length function enforcing that the length of the boundary is minimal; the second is a gradient-regularized area function ensuring that the region has a uniform intensity; and the third was introduced to penalize the deviation from a signed distance function. The function was determined by the Euler-Lagrange minimization equation and was subsequently solved by finite difference schemes. The use of gradient information together with area information makes it robust to handle problems with weak boundaries, as in our study. For more mathematical details, readers are referred to the original paper.[15]

The program was applied to subimages of the originals to reduce the computational cost. A subimage of 256 × 256 pixels, as shown in Figure 1B, was first obtained by cropping an area containing the FAZ from the original illustrated in Figure 1A. We then enhanced the subimage through a Gaussian filter. A Gaussian filter is an effective smoothing filter for random noise reduction that can also lead to blurring. In practice, the standard deviation and the window size of a Gaussian filter should be chosen with care to balance noise reduction against image degradation. Their values are usually chosen from experience. Typically, a σ value between 1 and 2 is chosen. In this study, we chose a Gaussian filter $\sigma = 1.5$ with a window size of 3 × 3 because visually they generated sufficient enhancement for the subsequent analyses. The enhanced image is illustrated in Figure 2A. The edge indicator information, shown in Figure 2B, was then computed based on the calculation of the intensity gradient over the whole enhanced images. An initializing contour was manually placed inside the FAZ, as shown in Figure 2C, and iteratively moved by the program toward the desired boundary guided by the edge indicator information. The program terminated when a fixed iteration step of 1000 was reached. Figures 2D to 2F show the evolving boundary, denoted in red, after 100, 200, and 400 steps, respectively. Figure 2G illustrates the final segmentation result. The program was run five times with different initializations on each of the 26 images to evaluate reliability and repeatability.

Manual Delineation. Manual delineation of the FAZ was used as the reference standard for validation of the automated segmentation program. Training sessions on the interpretation of FAZ and how to trace the boundary were given before manual annotation to minimize the discrepancy of understanding and/or manner of drawing. The boundary of the FAZ in the FFA images was then manually delineated

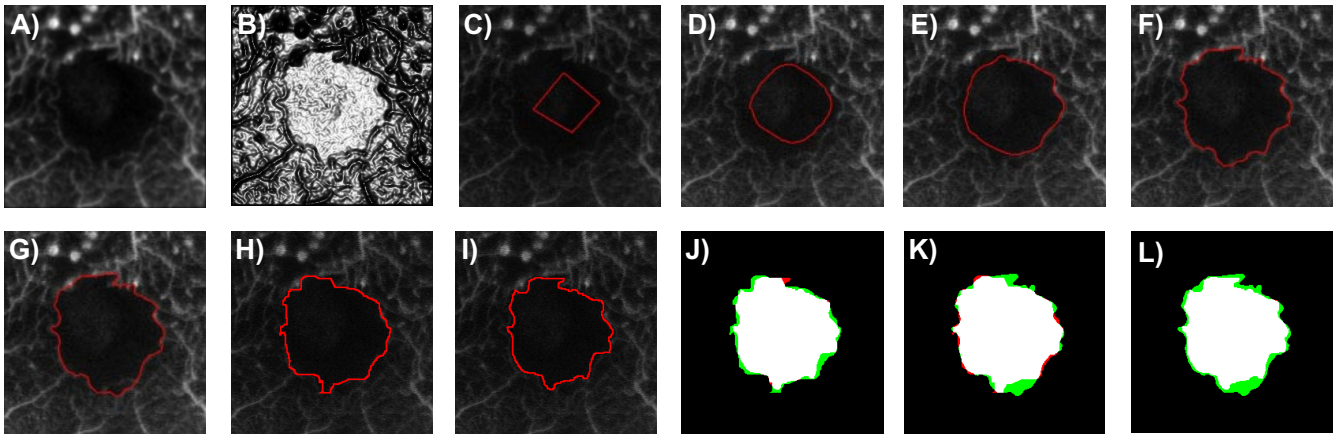

FIGURE 2. Illustration of the segmentation process. (**A**) Smoothed image ($\sigma = 1.5$) of Figure 1B. (**B**) Edge indicator. (**C**) Initializing curve. (**D**) Iteration 100 steps. (**E**) 200 steps. (**F**) 400 steps. (**G**) Final result. (**H**) Manual delineation of FAZ outline by expert 1. (**I**) Manual delineation of the FAZ outline by expert 2. (**J**) Overlapping between experts 1 and 2. (**K**) Overlapping between automation and expert 1. (**L**) Overlapping between automation and expert 2. (**J–L**) *White*: common area; *red*: portion of FAZ detected by the first method only; *green*: portion of FAZ detected by the second method.

by two experts (JSG, ANS) independently. Figures 2H and 2I illustrate annotations of the FAZ in Figure 1B by each expert.

Quantitative Measures. Two quantitative measures were used to assess the performance of our automated program: the size of the FAZ and the overlapping ratio (both derivable from the detected borders).

In the original ETDRS grading system, the size of the FAZ was defined by its greatest linear diameter.[10] However, we followed the definition used in previous studies[18–20] by which the FAZ was measured as an area calculated by multiplying the dimension of a pixel and the total number of pixels within the defined borders. Comparing the size of the FAZ measured only in different manners is not sufficient for assessing the agreement within them (i.e., in the worst scenario, two methods may yield identical quantitative values though the areas determined by them might be in totally different places). As a result, we adopted an overlapping ratio for measuring the agreement between different techniques.

The overlapping ratio, or Jaccard index, is defined as the ratio of intersection ($A \cap B$) of two measures, A and B, over their union ($A \cup B$). The value of this ratio ranges from 0 to 1, with 0 indicating no overlap at all and 1 indicating perfect agreement between both measurements. The size of the FAZ and the overlapping ratio were computed by programs written in commercial software (MatLab R14; The MathWorks Inc.). Figures 2J, 2K, and 2L illustrate the overlapping between 2 experts (JAG, ANS), automation versus expert 1, and automation versus expert 2, respectively. White indicates pixels detected

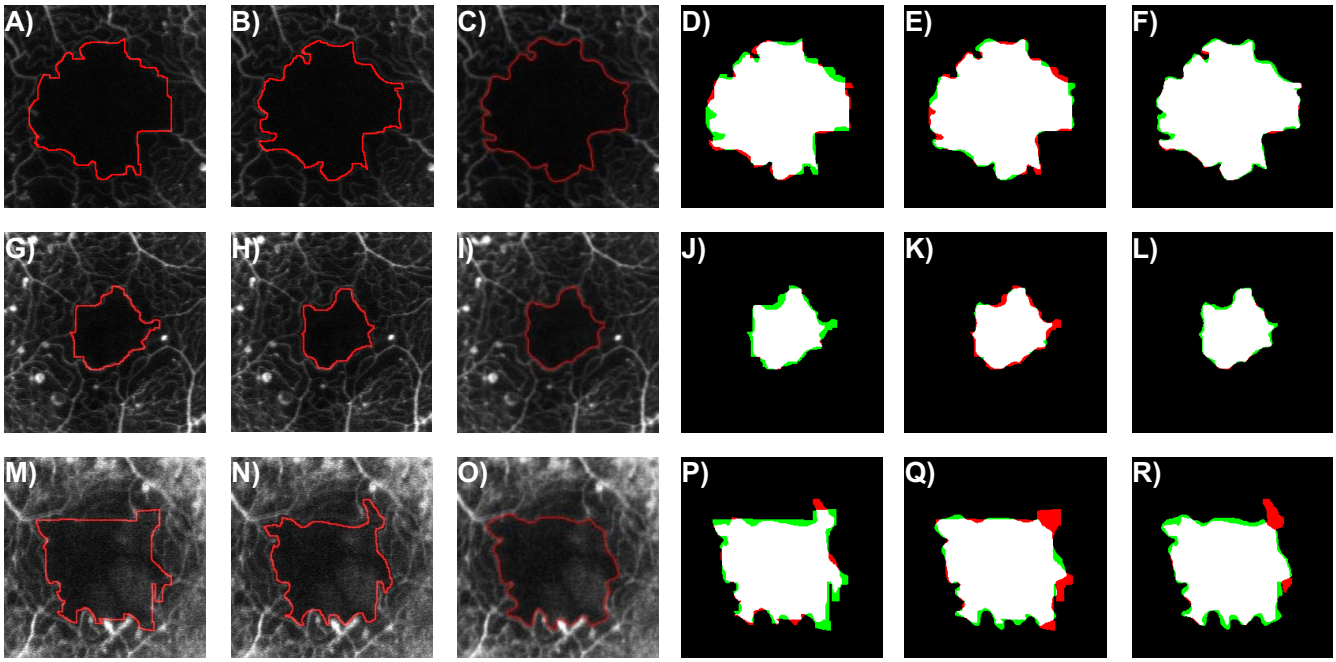

FIGURE 3. Comparison between the areas automatically detected by the program with those manually delineated by two experts. Each row represents a single case. *Left* to *right*: delineation of FAZ outline by expert 1 (**A**, **G**, **M**). Delineation of the FAZ outline by expert 2 (**B**, **H**, **N**). Result of the program (**C**, **I**, **O**). Overlapping between areas delineated by expert 1 and expert 2 (**D**, **J**, **P**). Overlapping between areas detected by the program and those delineated by expert 1 (**E**, **K**, **Q**). Overlapping between areas detected by the program and those delineated by expert 2 (**F**, **L**, **R**).

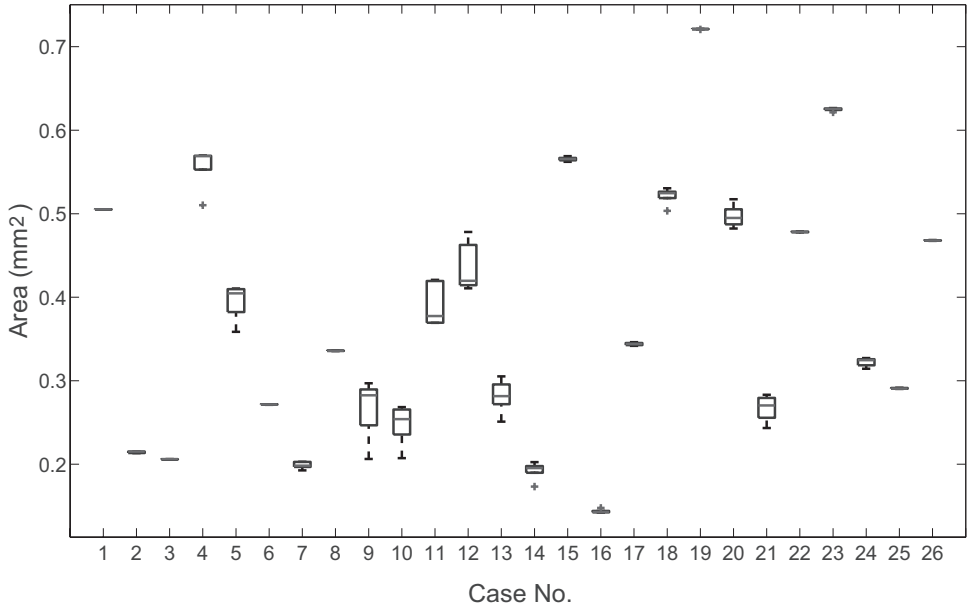

FIGURE 4. Box plot to illustrate variations of the FAZ area in 26 patients as determined by automated segmentation technique.

by both methods; red represents pixels detected by only the first method; and green represents pixels detected by only the second method. Figure 3 illustrates the comparisons in three or more cases.

Statistical Analyses

One-way analysis of variance (ANOVA) was undertaken to evaluate the repeatability of the proposed technique for the following criteria: overlapping ratios between manual delineation by expert 1 and automation; overlapping ratio between manual delineation by expert 2 and automation; and FAZ size. One-way ANOVA was also used to evaluate the difference in FAZ size determined by different methods. Linear regressions were calculated to evaluate the relation between FAZ sizes determined by different techniques. For all tests, $P < 0.05$ was considered statistically significant, and correlation coefficients with 95% confidence intervals were calculated to estimate agreement between different methods.

RESULTS

One-way ANOVA indicated no statistically significant difference among the five repeated tests in terms of overlapping ratio between automation and manual delineation by expert 1 ($F_{crit} = 2.444$, $F = 0.058$, $P = 0.993$) and between automation and expert 2 ($F_{crit} = 2.444$, $F = 0.036$, $P = 0.997$). Figure 4 demonstrates that the differences in FAZ size among the five different tests were relatively small. One-way ANOVA also showed that there was no statistical difference in FAZ size determined by five repeated tests ($F_{crit} = 2.444$, $F = 0.014$, $P = 0.999$). These tests demonstrated that the repeatability of the program was satisfactory. Furthermore, they provided justification for us to arbitrarily choose any of the tests representing the results of the automation for the further performance analyses. Table 1 presents the results of overlapping ratios repeated five times with different initializations for manual delineation by expert 1 versus automation.

Table 2 presents FAZ size detected by[1] three methods. One-way ANOVA showed no statistically significant differences among assessments of FAZ size determined by expert 1, expert 2, and automation ($F_{crit} = 3.12$, $F = 0.075$, $P = 0.928$). Table 3 presents the overlapping ratios for the three methods. One-way ANOVA was conducted to assess the differences among the overlapping ratios determined by expert 1, expert 2, and automation. Again, no statistically significant differences were found among these three methods ($F_{crit} = 3.12$, $F = 0.987$, $P = 0.377$). Tests on FAZ size and overlapping ratios demonstrated that the results of automation techniques were comparable to those of manual techniques.

Finally, we tested the linear correlations among manual and automated results. Figure 5 shows three regression curves: manual delineation by expert 1 = 0.939 × automation + 0.033 mm^2; manual delineation by expert 2 = 0.865 × automation + 0.044 mm^2; and manual delineation by expert 2 = 0.887 × manual delineation by expert 1 + 0.027 mm^2. Linear regression analyses show that the correlations between two experts and those between automation and expert are strongly statistically significant. Poisson correlation coefficients were 0.916

TABLE 1. Overlapping Ratios of Five Tests with Different Initializations

Test	Average	Variance
1	0.783	0.011
2	0.784	0.01
3	0.791	0.01
4	0.792	0.01
5	0.782	0.012

TABLE 2. Description of the Size of FAZ (mm^2)

	Expert 1	Expert 2	Automation
Minimum	0.210	0.195	0.142
Maximum	0.763	0.712	0.721
Mean	0.380	0.364	0.369
Standard deviation	0.158	0.146	0.154

TABLE 3. Description of the Overlapping Ratios of FAZ

	Automation vs. Expert 1	Automation vs. Expert 2	Expert 1 vs. Expert 2
Minimum	0.494	0.612	0.597
Maximum	0.926	0.947	0.905
Mean	0.783	0.771	0.807
Standard deviation	0.105	0.102	0.074

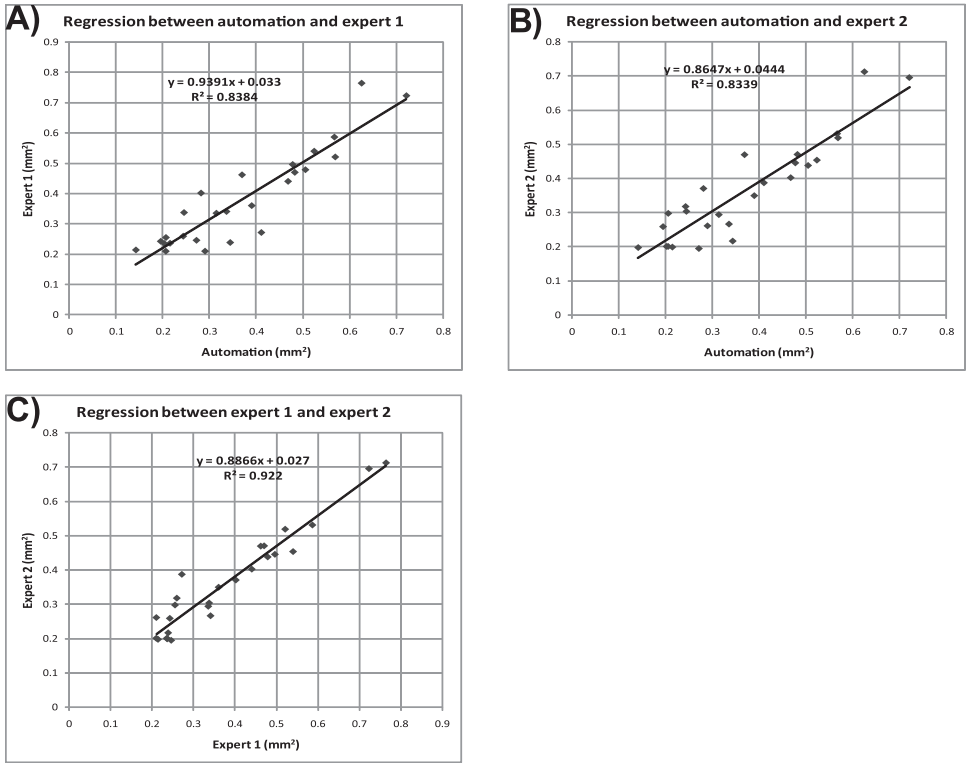

FIGURE 5. Linear regression analyses. (**A**) Linear regression between manual delineation by expert 1 and the program. (**B**) Linear regression between manual delineation by expert 2 and the program. (**C**) Linear regression between manual delineation by experts 1 and 2.

(95% confidence interval [CI], 0.818–0.962) between expert 1 and automation, 0.913 (95% CI, 0.814–0.961) between expert 2 and automation, and 0.96 (95% CI, 0.912–0.982) between expert 1 and expert 2.

DISCUSSION

We present an evaluation of the performance of a novel computerized automated segmentation technique specifically designed for quantification of the FAZ. The concordance between computerized measurement and manual delineation by clinicians was found to be 91%. The computer program showed high reproducibility, with the inference that such an automated technique may be useful in characterizing the size and contour of the FAZ in diabetic maculopathy.

To our knowledge, there are only three groups who have described automated FAZ segmentation.[12–14] Conrath et al.[14] described an automated technique for measurement of the FAZ. However, their technique produced a smaller FAZ area than that measured by clinicians, and the difference was statistically significant. Our principal objective was to develop a robust segmentation system that could accurately and reproducibly map out the FAZ boundary. Our approach benefited from rigorous level-set methods that are superior to image analysis techniques used in the previous studies.[12–14] The developed program yields high accuracy and reproducibility compared with its forerunners in the literature. However, as is the case with previous work, the present computerized technique requires manual initialization. Nevertheless, there is potential for further improvement such that operator input to start the program is not necessary. There are a number of strategies that may be used to allow self-initialization by the program. For example, the location of fovea could be estimated by using landmarks such as the optic disc and the retinal vasculature. If this facility can be introduced, the system may have an application in the computerized grading of clinical images.

On average, after manual initialization, it takes approximately 66 seconds for the program to complete a single segmentation task. Clearly, there is scope for increasing the speed at which an image is read by the program. Although we were aware of this need for computational efficiency, our central aim was to focus on accuracy and reproducibility. Although consistent and reliable performance was observed in this study, it should be noted that the formulation of the method itself was not entirely optimal in a mathematical sense, a factor that may lead to variation in segmentation with different initializations. Modification of our method to embrace the latest variational segmentation models using fast implementation of global minimization strategy[21] could overcome these limitations and improve computational efficiency.

Furthermore, comparison of our segmentation technique with that of others is challenging because of fundamental variations in data, such as image quality and extent of ocular disease. For instance, the correlation coefficient between our computer program and manual delineation was lower than that reported in a study that preferentially used normal fundi to examine intraobserver (0.9963) and interobserver (0.9945) variability between clinicians.[22] Notably, assessment of the FAZ outline is easier in normal fundi because the vascular architecture has not been obscured by disease. In addition to variation in the amount of disease, many technical factors preclude a direct comparison of our technique with techniques described in the current literature. As a case in point, one recent computerized method used pixels to quantify FAZ size, but the dimensions of the pixel were not described.[14]

Traditionally, image quality has been of overriding importance in the segmentation process. Increasing use of confocal laser ophthalmoscopes, such as the HRA2 system, has led to better image capture compared with those obtained with conventional fundus camera. Therefore, the selection of a good quality image for analysis no longer appears to be a prerequisite in practice. We used FFA images of a quality

deemed adequate for clinical assessment because our aim was to produce a program that could be implemented in a typical clinical setting. In contrast, similar studies have preferred high-quality FFAs.[5,14,23] Therefore it is likely that our program would produce even better results if only good quality images are selected for analysis. However, in routine clinical practice, image quality is affected by various factors, including media opacity and poor patient cooperation. When the images are suboptimal, even experienced clinicians cannot make decisions with confidence. Such difficulty will also affect computerized programs and limit their reliability. In subsequent work, therefore, we aim to develop techniques (such as refinement of the algorithmic formula) that preprocess the image before segmentation analysis is begun.

We found a larger mean FAZ size in patients with diabetes than was reported in healthy subjects from three studies.[18–20] In comparison, our mean FAZ size was smaller than that documented in a fourth study involving healthy subjects.[24] The finding of an enlarged FAZ size was consistent with our original expectations, which were based on the pathophysiological basis of diabetic maculopathy. Microangiopathy is observed as a loss of the perifoveal vasculature and the accompanying enlargement of the central avascular zone. Accordingly, many studies have used FAZ size as an indicator of the extent of ischemic maculopathy in diabetes.[4,5,18] FAZ size is also increased in other forms of ischemic maculopathy. In sickle cell disease, it has been noted that patients with maculopathy have statistically larger FAZ sizes than do healthy controls,[1] and a similar finding has been reported in patients with central retinal vein occlusion.[25] These studies highlighted the potential of our program for the assessment of a wide range of ischemic maculopathy. One study examined FAZ diameter and perifoveal intercapillary area and suggested that macular ischemia may cause disturbances in neuronal function that precede the deterioration of visual acuity.[6] It is possible that measurement of the FAZ area may permit characterization of a critical hypoxic threshold for visual loss that is relevant to various disease processes.[2]

The association between enlarged FAZ and reduced visual acuity has been reported in other studies.[2,20,26] For example, a positive correlation between severity of FAZ damage and best-corrected visual acuity has been documented.[27] Despite these compelling reports controversy remains. One study in 23 diabetes patients with cystoid macular edema noted a lack of significant correlation between FAZ size and visual acuity.[28] Another group reported a negative correlation between FAZ size and perifoveal intercapillary area size when these two parameters were compared against contrast sensitivity in 20 diabetes patients without clinically significant macular edema.[3] Moreover, there was a significant correlation between increasing FAZ diameter and increasing implicit time of the innermost concentric rings and of the third concentric ring in the first-order kernel of multifocal electroretinography.[6] It is likely, however, that FAZ enlargement is associated with loss of visual acuity resulting from loss of perfusion to neuronal tissue. Therefore, FAZ characterization in the diabetic eye is a clinically relevant exercise.

Our method has particular application within research given that patients with diabetic ischemic maculopathy are often excluded from therapeutic studies. By providing a tool for improved FAZ measurement, this technique would theoretically facilitate the correlation of such a baseline clinical parameter with the response to treatment. Yet another use may be in the measurement of ischemic zones in extramacular areas of the fundus. By calculating the total area of nonperfusion, the clinician may gain a better strategy for assessing the likelihood of ischemia-related complications in diseases such as central retinal vein occlusion.

In the present study we have observed that an automated computer program can accurately and reproducibly measure FAZ. This technique has the potential to serve as a powerful tool in the automated assessment and grading of images in a routine clinical setting.

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