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Automated detection and quantification of microaneurysms in fluorescein angiograms*

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Abstract. Fluorescein angiograms from diabetics were digitised for analysis using digital image-processing techniques. Computer algorithms were written to detect and count microaneurysms present in the images. The accuracy, speed and reproducibility of the technique were assessed and compared with those of manual counts made by clinicians from both digitised and analogue images. Free-response ROC (receiver operating characteristic) curves were used to assess the performance of both the clinicians and the computer by comparing the results with "gold standards" compiled from prints of the original fluorescein angiograms. The computer performed as well as the clinicians when the latter were analysing the digitised images (512 × 512 pixel resolution), but only when one image was acquired at 4 times this resolution did the computer's performance match that of the clinicians analysing the analogue image. The automated technique was more reproducible than the manual method.

Introduction

Diabetes is the commonest cause of blindness in the working-age population in the United Kingdom. A number of retinal lesions are associated with the disease, including microaneurysms, oedema, hard exudates and haemorrhages. The presence of microaneurysms is the first unequivocal indication of early diabetic retinopathy, and there is a positive correlation between their numbers and the progression of the disease [12, 13].

Although microaneurysms can be seen on colour fundus photographs, the lesions are best visualised on fluorescein angiograms [6]. For example, the red reflex of the retina reduces the definition of small microaneurysms in colour fundus photographs, making it difficult to differentiate between small haemorrhages and microaneurysms.

For manual counting of the microaneurysms in a fluorescein angiogram, the negative has to be projected onto a screen. Manual counting procedures [2] are laborious, time-consuming and subject to human error. Digitisation of the fundus images enables the application of computer processing techniques to the images, first to discriminate automatically the microaneurysms from the other features and second to count the number of microaneurysms present. Computers are well suited to problems involving the extraction of quantitative information from such images because of their ability to process data in fast and efficient manner with a high degree of reproducibility [7, 8, 15, 17].

In the present study, computer algorithms were developed for the detection and quantitation of microaneurysms in digitised fluorescein angiograms and the effectiveness of the technique was assessed. A comparison was made between manual counting from both digitised and analogue photographs and the performance of the automated technique. Free-response ROC (receiver operating characteristic) curves were used to assess the performance of both man and machine.

Materials and methods

Image processing

Six fluorescein angiograms were collected from six clinically proven diabetics. For digitisation of the images, each fluorescein angiogram negative was projected onto a screen using a Slidex projector. A monochrome CCD (charge-coupled device) camera (JVC TK-5310) mounted opposite the screen was used to capture each image. The camera was connected to a frame-grabber (Data Translation DT-2861) that stored the image as a 512 × 512 picture-element (pixel) array, each element being assigned an intensity grey level of 0–255, depending on the intensity of the projected angiogram at

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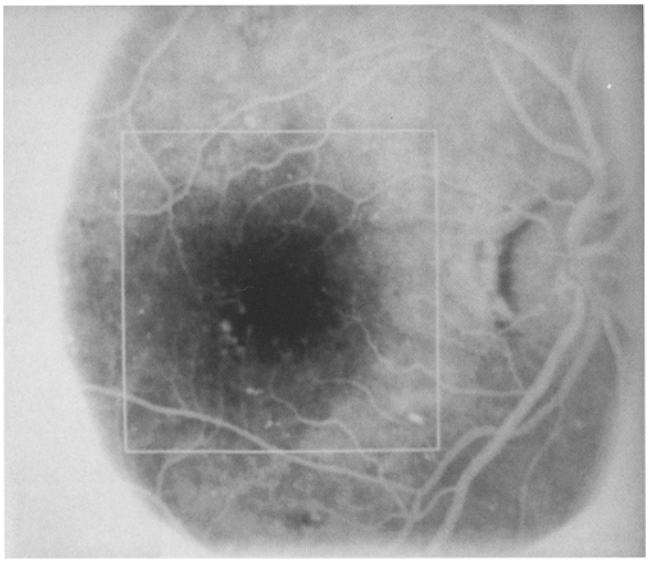

Fig. 1. Digitised fluorescein angiogram of a diabetic patient, showing the presence of microaneurysms. The ROI enclosed by the white box centered on the fovea encompasses 192×256 pixels

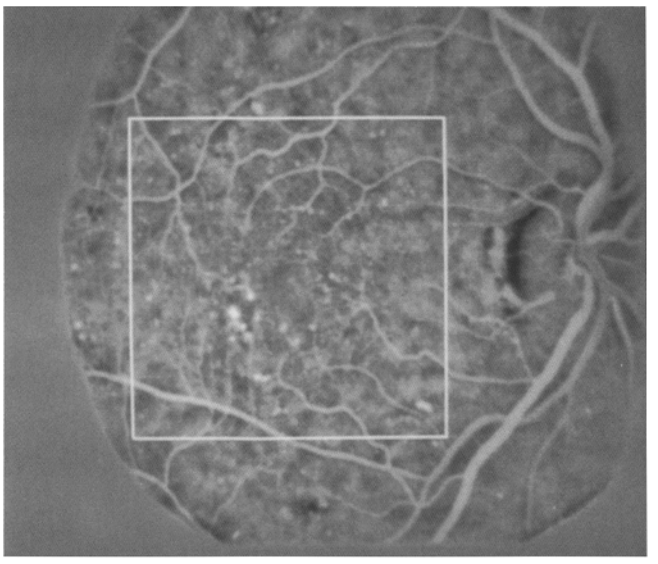

Fig. 2. The pre-processed image following radiometric normalisation and shade correction. The position and size of the ROI are the same as those shown in Fig. 1

that point. The frame-grabber was controlled by a personal computer (IBM PC-AT clone) that was used in conjunction with an auxiliary frame-processor (Data Translation DT-2858) to carry out standard image-processing operations. The digitised images were viewed on a high-resolution colour monitor. Figure 1 shows a digitised fluorescein angiogram with a region of interest (ROI) centered on the fovea that encompasses 192×256 pixels. The image intensity was inverted by the computer such that areas of hyperfluorescence were light as compared with darker areas of hypofluorescence.

Image pre-processing

It was necessary to apply a number of pre-processing steps to the digitised images prior to their analysis. Any over- or underexposure of the fluorescein angiograms resulted in a change in both the average intensity and the contrast of the developed film. When fluorescein angiograms acquired on different occasions are analysed, discrepancies in contrast between the various images may invalidate any quantitative comparison. Thus, the digitised images were normalised using a radiometric correction factor [1] such that they all exhibited the same range of pixel grey levels and the same average grey level. Unwanted changes in intensity that occurred across the image due to the photographic process and to choroidal fluorescence were removed by a process known as shade correction [10, 19]. The image was then suitable for analysis (Fig. 2).

Microaneurysm detection

The microaneurysms were detected following the application of matched filters to the image. Matched filters are designed to assume the same size and shape as the object to be detected [5]. Digitally, the filter comprises a square matrix of numbers whose values correspond spatially to the grey levels of the desired object. Having studied the intensity cross-sections of microaneurysms from various digitised fluorescein angiograms, we concluded that the lesions could be approximated to a two-dimensional Gaussian (or bell-shaped) distribution. Microaneurysms exhibiting sharp peaks could be modelled to Gaussian distributions using low values of sigma ($\sigma = 0.5$–1.5 pixels) and those displaying gently sloping peaks could be modelled at higher values of sigma ($\sigma = 2$–4 pixels). Similarly, microaneurysms exhibiting large diameters could be modelled using matched filters measuring 9×9 or 7×7 pixels and smaller ones comprising 5×5 or 3×3 matrices. Ideally, a complete range of matched filters of various sizes and sigma values would be applied to the image to optimise the detection of all of the various sizes and shapes of microaneurysms present in the image. However, this would be computationally intensive and would result in an unacceptably long processing time. To maximise the speed of the processing, one 5×5 pixel filter of $\sigma = 1$ was used to detect microaneurysms of all sizes.

The image was convolved with the filter and the output at each position was a measure of the correlation between the matched filter and the shape of the feature represented by the image pixels at that location (Fig. 3a). Thus, the intensity (grey level) of the pixels in the filtered image was related to the likelihood of a microaneurysm being present at that location. By the application of a suitable grey-level threshold to the resulting image, the microaneurysms were separated from the other features. To detect microaneurysms whose size and shape were significantly different from those of the matched filter, a relatively low threshold had to be used (Fig. 3b). However, this resulted in the appearance of false positives on the vessels due to the uneven distributin of fluorescein in the vasculature. To eliminate this type of false positive, additional processing had to be performed to delineate the vasculature.

Vasculature processing

To enable the separation of vessels from other features in the image, a "vessel mask" was derived from a thresholded image obtained by convolving the original image with a 5×5 matched filter of $\sigma = 2$. This sigma value was chosen to maximise the response to vessels rather than to microaneurysms. A low threshold level was applied to the filtered image such that vessels were included but the resultant image also displayed microaneurysms and spurious background features. Various size and shape algorithms [18] were then applied to this image such that objects exhibiting a relatively high degree of circularity (including microaneurysms and any other blob-like features) were rejected, leaving long, linear structures that represented the main retinal vessels (Fig. 3c). This vessel mask was superimposed on the original thresholded image (shown in Fig. 3b) such that pseudo-microaneurysms whose positions coincided with

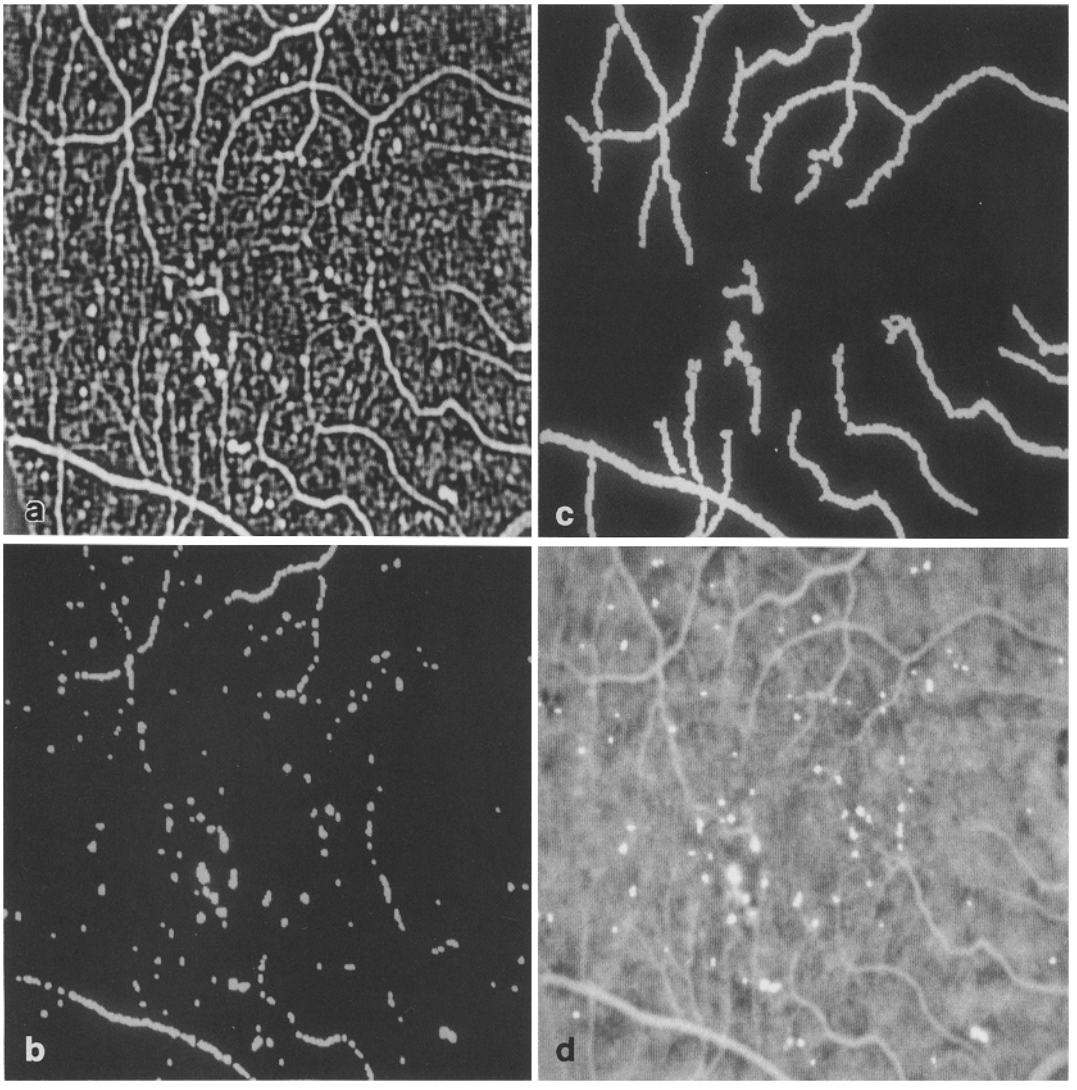

Fig. 3a–d. The ROI at four stages of processing. **a** The result of applying a matched filter to the pre-processed image. **b** Thresholded image showing detected microaneurysms and vascular false positives. **c** Delineated vasculature ("vessel mask"). **d** Detected microaneurysms superimposed on the shade-corrected image

any part of this vessel mask could be removed. The computer automatically counted the number of microaneurysms remaining. The microaneurysms detected are shown in Fig. 3d.

Assessment

To assess the accuracy, reproducibility and speed of the computer technique, six fluorescein angiograms were analysed. Prints were made of each of the six angiograms at a magnification of 7 times the size of the original 35-mm negative. Prints were also made of the six digitised images. A transparent film with the ROI (corresponding to that used in the computer analysis) marked on it was overlaid on each print. Five experienced clinicians (two consultants, three registrars) were asked to mark the positions of any microaneurysms on the overlay in ink, with each clinician being unaware of the responses of the others. They were asked to analyse the digitised images first and the analogue images 1 week thereafter.

The correct locations of the microaneurysms were established following a thorough examination of the analogue prints by two of the authors, comparison of their results and subsequent discussion on the inclusion or exclusion of any ambiguous lesions. Each clinician's results were scored in terms of the percentage of true-positive responses (sensitivity) and the number of false-positive responses. The same digitised images were analysed by the computer. The numbers of true and false positives were recorded as the grey-level threshold value was varied.

In addition, to examine further the effect of the digitised image resolution on the performance of the computer technique, one fluorescein angiogram was acquired with the camera zoomed-in by a factor of 2, giving an image that covered only one-quarter of the angiogram but whose resolution was 4 times that of the previous acquisition. Matched filters encompassing 5×5, 7×7 and 9×9 pixels were used to detect the microaneurysms in a sub-section of the ROI and were compared with the clinicians' results for the corresponding sub-region. The effect of the re-acquisition of an image on the variability of the computer's results was measured by comparing the microaneurysm counts from 14 different ROIs obtained on 5 separate occasions, disregarding false positives and false negatives.

Results

The ability of clinicians and computers to detect microaneurysms is assessed in terms of both the number of

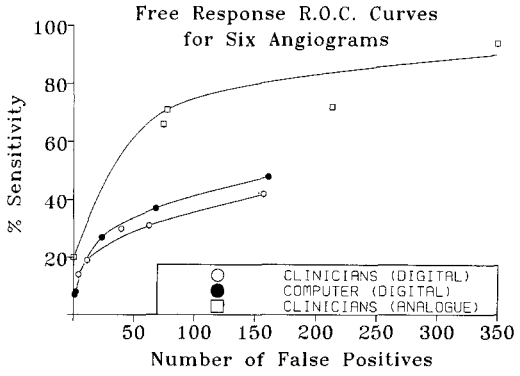

Fig. 4. Free-response ROC curves for the clinicians' analyses of the analogue and digitised images and for the computer's analysis of the digitised images. Each *open circle* or *open square* represents the results obtained by one clinician, summed over the six images. The computer curve (*solid circles*) was produced by varying the final threshold applied to the matched filtered images. The total correct number of microaneurysms was 541

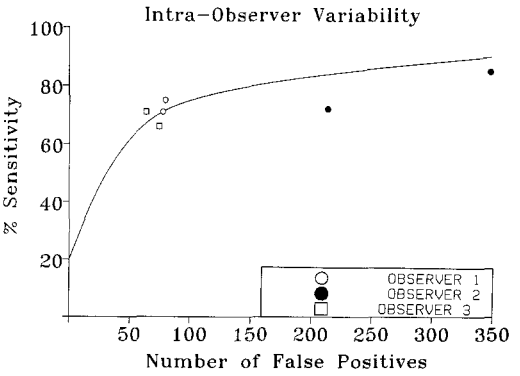

Fig. 5. Graph illustrating intra-observer variability. Each pair of points corresponds to the results obtained by one clinician on two separate occasions. The curve was taken from Fig. 4 (clinicians' analyses of analogue images)

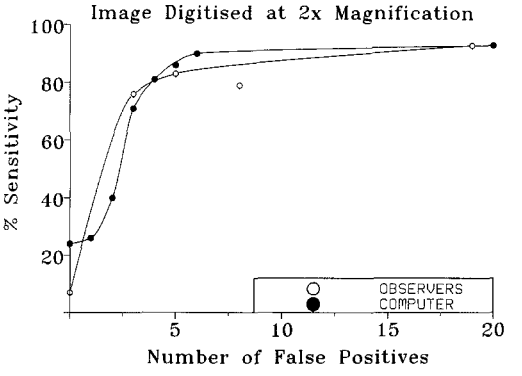

Fig. 6. Free-response ROC curves for the computer's analysis of the image magnified 2× before digitisation (≡4× the previous resolution) and for the clinicians' analyses of the corresponding sub-section of the ROI

"correct", i.e. true-positive, responses and the number of "incorrect", i.e. false-positive, responses. It is well recognised that clinicians can only achieve an increase in the number of their correct responses at the expense of obtaining more incorrect responses as well [14]. The plot demonstrating the manner in which true and false positives vary together is known as the receiver operating characteristic (ROC) curve. In an experimental situation such as this, in which the maximal number of false positives is not known, it is obviously not possible to calculate the false-positive rate as a percentage of the total. Instead, the ROC curve is plotted as the true-positive response rate (%) against the number of false-positive responses; this is the so-called free-response ROC curve [4].

Figure 4 shows the free-response ROC curves for the clinicians' analyses of analogue and digitised images and for the computer's analysis of the digitised images. Although the true- and false-positive response rates obtained by the clinicians showed inter-individual variation, they fell on the same free-response ROC curve. This showed that there was no difference in the clinicians' potential ability to differentiate microaneurysms but that their likelihood of reporting the presence of a microaneurysm varied. There was no difference between the performance of the computer and that of the clinicians when the latter were analysing the digitised images, but the clinicians performed much better using the analogue images, i.e. for any false-positive rate they achieved a much higher true-positive rate (sensitivity).

The graph in Fig. 5 shows the intra-observer variability, with each pair of points representing the results obtained by one clinician on two separate occasions. Although two of the clinicians were consistent in their analysis of the images, the analysis by the third clinician (solid circles) showed considerable variation between the two readings. The difference between the digitised and the analogue images was most likely due to the degrading effect of the rather coarse sampling of the digitising process. Figure 6 shows the results obtained by the clinicians and the machine when the computer analysed a magnified angiogram (4 times the previous digitised resolution). In this case, the computer performed as well as the clinicians did.

Discussion

A number of protocols have been described for manual counting of microaneurysms from both colour fundus photographs [12] and fluorescein angiograms [2]. The image is divided into a number of sub-fields to enable the counting of large numbers of lesions. The technique of Baudoin et al. [2] involved the projection of fluorescein angiograms magnified 13 and 17.5 times with respect to the original negatives, following which observers were asked to classify the microaneurysms as being either "definite" or "possible" according to their size. The inter- and intra-observer variability was measured in terms of two separate criteria: microaneurysm count and microaneurysm position. Although the correlation between inter- and intra-observer counts was satisfactory ($r > 0.9$), it gave no measure of the sensitivity or specificity of the observers. Indeed, the reproducibility of the

results obtained for microaneurysm positions (measured as the ratio of microaneurysms present in the *same* positions in both readings to the total count for both readings) was 60%–71%, depending on the quality of the images. This implied that there could have been a significant false-negative and/or false-positive rate, since the readings represented samples from the same population.

Baudoin et al. [3] subsequently described a fully automated method that used projected fluorescein angiograms magnified 150 times and digitised at a resolution of 256×256 pixels; the average diameter for the microaneurysms was 15 pixels as compared with 5 pixels in the present study. This comparatively large magnification factor resulted in each angiogram having to be divided into 25 regions for digitisation and subsequent processing. To evaluate the technique, these authors compared the numbers of microaneurysms counted by the computer with the counts made by trained technicians and clinicians, again giving no indication as to the proportion of correct and incorrect responses. To obtain some measure of the sensitivity and specificity of the automated method, each of the computer's results was superimposed on the original digitised region; the technician who had shown the best reproducibility then compared the positions of the microaneurysms he had found with those detected by the computer, and vice versa. This served to establish only the number of microaneurysms missed by each method rather than the actual false-positive and false-negative rates, since neither method could be called the "gold standard".

It must be noted that any attempt to construct a "gold standard" is difficult in cases in which very small, leaky or low-constrast lesions are not easily differentiated from background fluorescence, nearby vessels or any other features immediately adjacent to the lesions. Such a situation results in conflicting opinions regarding the presence or absence of such lesions. What is the correct answer?

The digitisation resolution of the system described in this paper was clearly an important factor in the sensitivity of the automated method, and this can be shown by a comparison of the graphs in Figs. 4 and 6. It is clear that the computer method is as good as the observers' analyses of an analogue image when the former uses an image digitised at 4 times the previous resolution. Technology exists that enables the acquisition of images using a CCD camera at a resolution of $1{,}024 \times 1{,}024$ pixels, resulting in greater sensitivity for the automated technique, which has the potential to perform as well as human observers. The reproducibility of the computer method would certainly be better than that of any manual method, which inevitably suffers from human fatigue and inter-observer variability. One of the benefits of the computer's "objectivity" is that it uses only information extracted from the area it has been instructed to analyse, unlike the clinician, who may receive cues from other regions of the image. The division of the image into smaller sections as an alternative way of achieving greater resolution should be avoided, since this creates problems involving the registration of sub-images. Due to the small size of microaneurysms, any registration technique [11] must be highly accurate if the lesions are not to be missed or duplicated.

The amounts of time taken by the computer and the clinicians to analyse the images in the present study were similar (neither type of analysis took longer than 5 min) and were considerably lower than those involved in Baudoin's manual or automatic methods (30–40 min). The speed of the automated method was limited by the processing power of the computer hardware and would be improved by the use of a more powerful machine. In addition, we propose that the images be directly acquired from the patients so as to avoid the numerous non-linearities inherent in the photographic process, which diminish the quality of the fluorescein angiograms, especially when quantitative information is to be retrieved.

The further development of this technique will provide a useful clinical tool for documentation of the progression of diabetic retinopathy. Macular oedema [16] and macular non-perfusion [9] have been detected and quantified by computer processing of fluorescein angiograms, and hard exudates have been detected and quantified from colour fundus photographs [19]. The quantification of these lesions furnishes important clinical information regarding the progression of diabetic retinopathy and, together with microaneurysm counts, should be collected as routine data for analysis of the patient's fluorescein angiogram and colour fundus photograph.

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