A Modified Semiautomatic Method for Measurement of Hyperfluorescence Area in Fluorescein Angiography

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Abstract

**Purpose:** To offer a semi-automatic algorithm to determine the area of angiographic hyperfluorescence

**Methods:** The proposed algorithm included wavelet filter, histogram equalization and modified Otsu thresholding. Hyperfluorescent area of 30 angiographic images obtained from patients with leaking choroidal neovascularization (CNV) and diabetic retinopathy were evaluated and the results were compared with those obtained by an expert ophthalmologist.

**Results:** The best wavelet filter was determined. Quantitative assessment of the hyperfluorescent area showed a mean error of 0.12±0.59 square millimeters. There was no significant difference between algorithm-measured and ophthalmologist-measured area.

**Conclusion:** The proposed algorithm may be used to accurately measure area of hyperfluorescence.

**Keywords:** Wavelet, Age Related Macular Degeneration, Diabetes, Modified Otsu, Hyperfluorescence Region


Introduction

Age related macular degeneration (AMD) and diabetes mellitus are two major causes of visual loss throughout the world.1,2 In advanced AMD, neovascularization of the choroid (CNV) causes extravasation of fluid, lipid, and subretinal accumulation of blood and lipid. Similar mechanism, although, at the level of retina and preretina causes accumulation of intraretinal and preretinal exudates and blood. Also, hyperpermeability of the vessels in diabetic retinopathy induces intraretinal edema and exudation. Fluorescein angiography (FA) helps to determine the vascular pattern and their competency. Vascular leakage in diabetic retinopathy and AMD associated with CNV appears as hyperfluorescent areas in FA. Determining the extent of hyperfluorescence is important in management and follow-up of the disease.3
Automatic measurement of the hyperfluorescent area may enhance the accuracy of the results and feasibility of the measurements. Also, for assessing the homogeneity of the patient samples and monitoring the efficacy of a therapy under investigation, in clinical trials, a uniform image analysis system is necessary.

Several studies reported various automatic measurement methods for determining the hyperfluorescent area. These methods include pixel-by-pixel basis analysis of the sequential images, comparison with the normal background, comparing the leakage area to the disk area, thresholding, evaluating the hyperfluorescent change with Acetate plates, averaging and alignment, using Sobel filter and gradient vector flow snake, have several drawbacks, mainly, failure to report the statistical results, absence of quantitative evaluation and failure to compare the results with those obtained by an ophthalmologist. We evaluated the accuracy of the modified Otsu thresholding method accompanied by wavelet filter for measurement of the area of hyperfluorescence due to CNV or diabetic retinopathy in different phases. These cases were diagnosed by an ophthalmologist.

Methods
We obtained 30 FA images in different phases from patients with active CNV or diabetic retinopathy. These images were saved with “Tiff” format. An expert ophthalmologist selected the area to be measured by algorithm [Region of interest (ROI)]. The designed algorithm with three main steps was applied to the selected ROI. A flowchart of the hyperfluorescence region segmentation in retinal angiography images is given in figure 1.

Accuracy of segmentation is affected by noise and nonuniformity of the images. Therefore, in this method we tried to decrease the noise and enhance uniformity of images.

Noise reduction
Reflected fluorescence of the surface of retina scatters incoherently in the internal space of eye. Therefore, images of retina have additive random noise. For this reason, wavelet filter was used to noise reduction. This filter reduces noise in three dimensions; horizontal, vertical and diagonal.

In this paper, to determine appropriate wavelet filter, different types of wavelet filters were applied to angiographic images. Applied wavelet filters on images included Daubechies, Coiflets and Biorthogonal. To determine proper filter wavelet peak signal to noise ratio (PSNR) was used. PSNR obtained by calculating 

\[
PSNR = 10 \log_{10} \left( \frac{255^2}{MSE} \right) \quad (1)
\]

where MSE is the mean squared error.

Enhancement of the image contrast
In these images, light intensity of images doesn’t have proper uniformity due to fluctuating laser beam and retinal curvature. The curvature of the retina causes to light intensity of FA images reduce from the center toward the corner. The changes in light intensity affect the thresholding determination. Therefore, histogram equalization method was applied before implementation segmentation step (Figure 3).

Segmentation
In this step to separate hyperfluorescence region from background, after noise reduction and histogram equalization, segmentation algorithm using thresholding method was implemented. Lin introduced fast algorithm based on Otsu method. So that in this paper modified Otsu algorithm was applied and evaluated on FA images.

In modified Otsu method, an image with L gray level is classified into two classes \( C_0 \) and \( C_1 \) at the gray level \( k \) where \( C_0 \) denotes the set of pixels with gray levels \( \{0,1,\ldots,k\} \) and \( C_1 \) denotes the set of pixels with gray levels \( \{k+1,k+2,\ldots,L-1\} \). Between classes variance (BCV) of the image is defined as below.

\[
\nu_B = \frac{1}{\omega_0(1-\omega_0)} \left( \omega_0 \mu_T - \mu_k \right)^2 \quad (1)
\]

Where \( \nu_B \) denotes the BCV; \( \omega_0 \), the zeroth-order cumulative moments for the classes \( C_0 \), respectively; \( \mu_T \) is the total mean gray level of the image; \( \mu_k \) denotes the first-order cumulative moment of the histogram up to the gray level \( k \).

Let us assume that BCV is differentiable with respect to gray level, and let the first derivative of the BCV be zero.
\[ f(k) = k - \frac{\mu_0 + \mu_1}{2} \quad (2) \]

Where \( \mu_0 \) and \( \mu_1 \) denotes mean gray levels for the classes \( C_0 \) and \( C_1 \).

Bisection method was used to solve the zero BCV-derivative equation. The method is based on the fact that when an interval \([k_0, k_2]\) has a root in it. An interval that includes the root is called an active interval. The active interval will be bisected again. This procedure iterates until no further bisection needs to be performed. In our case, the bisection starts by letting \( k_0 = 0 \) and \( k_2 = L - 1 \), and it takes \( \log_2 L \) iterations for the root solving. Among the midpoints generated, the one with minimal absolute value of the BCV-derivative function is selected as the optimal threshold.\(^{17}\)

After analyzing the images with the above-mentioned method we compared the result with those obtained manually by an expert ophthalmologist. The images were imported to an ImageNet2000\(^{\text{TM}}\) instrument (Topcon, Japan) and the hyperfluorescence region was marked by ophthalmologist and measured by the instrument software. The measurements were repeated again and the mean of 2 measurements was chosen for comparison. If the difference between the 2 measurements was more than 0.5 square millimeters the image was excluded.

The data were entered using SPSS (SPSS Inc., version 15) and analyzed using T-test.

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**Figure 1.** The hyperfluorescence region segmentation steps in retinal angiography images

**Figure 2.** The result of assessment of peak signal to noise ratio

**Figure 3.** Left: Original image, Right: After applying histogram equalization method
Results
Comparison of PSNRs of the images showed that filter wavelet Biorthogonal has the highest PSNR. Degree of freedom of this filter is greater than orthogonal wavelet filters. The main property of this filter is problem solving discontinuity of edges.

Segmentation algorithm was applied to 30 images. As is shown in figure 4 the hyperfluorescence region was separated automatically from background. Quantitative assessment the area of hyperfluorescence measured by software and ophthalmologist shows mean error 0.12±0.59 square millimeters and correlation between two groups is 0.98% (Table1).

There was no significant difference between software measurements (3.68±2.73) and ophthalmologist measurements (3.79±2.63) (t=1.147, df=29).

![Figure 4. Area measurement by suggested software](image)

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Discussion

FA is valuable for visualizing new vessels and is used to assess the response to treatment during clinical care and in clinical trials. Hyperfluorescence secondary to the fluorescent dye leakage from new and incompetent vessels gives us important information for management and follow up of patients with AMD and Diabetic retinopathy. The advent of optical coherence tomography (OCT) and precise measurement of retinal thickness and structure, has to some extent pushed FA into the background. However, FA should not be abandoned because it provides information different from that obtained using OCT. In patients with CNV, OCT provides a quantitative measure of retinal thickness and subretinal fluid. Reduction in subretinal fluid and retinal thickness during the treatment period suggests the decrease in the average amount of leakage over days to weeks before the next scan, but it does not provide a direct indication of the current amount of leakage. This information is provided by FA, which also demonstrates CNV lesion size, another outcome measure that cannot be assessed by OCT. Thus, FA and OCT provide complementary information.

Previous studies reported several methods for measurement of hyperfluorescent area.\(^4\) Philips et al\(^5\) evaluated the change in the hyperfluorescent area using a computer-based method which was able to compare the fluorescence activity within a ROI in the macular area to the same ROI in a later FA frame. Saito et al\(^6\) compared the fluorescence within a CNV to a normal background area. These studies, however, didn’t report quantitative measurements. Sickenberg et al\(^6\) compared the CNV surface to the disk area. The fluorescence intensity was measured comparing the grayscale fluorescence levels of the CNV classic pattern with the mean grayscale level of control areas. A combination of the CNV size parameter with the fluorescence contrast parameter was called integrated contrast (IC). The IC amplitude was then used to describe and compare the fluorescence before and after Photodynamic therapy (PDT). They didn’t report precise quantitative measurements (i.e. in millimeters). Berger et al\(^7\) used digital image processing for CNV area measurements, with normalization to optic disk area to control for magnification differences. They also measured relative CNV lesion fluorescence intensity by subtracting the background, but they performed subtraction on a single frame 600 seconds after fluorescein injection. This method was also criticized for using a uniform threshold for measurement of all images.

Bartsch et al\(^8\) used a software to average multiple images from a 10-20 image series. Image quality was graded by two masked observers. They devised a more quantifiable grading method by adding a variable amount of Gaussian noise to the improved image until the original and averaged image appeared equal. They reported mild to strong improvement of visualization of structures including borders of CNV that varied depending on the type and phase of the angiogram. They didn’t report the area of the hyperfluorescence, and only tried to detect the lesion margin.

Brankin et al\(^9\) used a combination of the ‘Sobel’ edge detection algorithm combined with thresholding produced the best qualitative segmentation, as verified by a trained ophthalmic grader. This report didn’t have any statistical results, moreover, the size of lesion wasn’t measured. Shah et al\(^10\) used a histogram analysis algorithm which was written by MATLAB software to select frames with appropriate exposure for grading. Once a grader outlined hyperfluorescent lesions on each original frame of a fluorescein angiogram, the image analysis software provided the lesion area in pixels. The grader also outlined the optic disk, and its area was determined. Lesion area was divided by the disk area for normalization to control for differences in magnification among the fluorescein angiograms. But, measurement of the size of the lesion wasn’t report. Kose et al\(^11\) introduced a method that at first extracts optic disk as a healthy area. So, histogram of the filtered image was used to extract optic disk. The maximum values of histograms were used to determine the position of the optic disk. If there was some degeneration around the optic disk, the result may be misdetected. To reduce the misdetection rate, an experimentally determined threshold value was used. The accuracy of segmentation was determined by
Threshold. The result also showed that the threshold may vary from image to image. They suggested that more clinical tests and experiments need to be done to choose more precise threshold value for an interested image. Then, blood vessels were also extracted and classified as healthy regions. In order to produce the final segmented image, the inverse image of the segmented image was generated as unhealthy region of the macula. The result showed that the suggested method leads to incorrect segmentation for a few cases.

**Conclusion**

In our study, modified Otsu thresholding was used for segmentation. In this method, it was assumed that BCV is differentiable with respect to gray level, and let the first derivative of the BCV be zero. The minimum of this equation shows best threshold. Wavelet filter was used to noise reduction. This filter reduces noise in three dimensions; horizontal, vertical and diagonal. Before applying the segmentation method, the intensity value was adjusted by histogram equalization which involves transformation of the intensity values. Eventually, for determining the accuracy of this algorithm, we compared the semi-automatic measurement results with those obtained by an expert ophthalmologist, and found no statistical difference. This method seems to have more precision than previous methods since the wavelet filter has more appropriate PSNR. Also, segmentation method of this algorithm has the capability to measure the distinct hyperfluorescent areas located apart from each other.

This study has some limitations. We did not report the inter-session and inter-observer measurements. Some areas with leakage-like patterns (i.e., window defects or staining) may be considered as leakage area erroneously and should be deleted from the measurement by the software operator. Despite these limitations, the accuracy of this method may make it to be applicable to the studies evaluating the therapeutic change in the fundus images. Also, recent progress in new angiographic-based methods like autofluorescence, may reappraise the need for automatic measurement of the changes in fundus images.

**References**