

# Segmentation in Blue Wave Fundus Autofluorescence Retinal Images

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**Abstract**— Segmentation of optic bundle and macula is the primary step in determining retinal diseases using image processing technique. An algorithm for the same is developed using MATLAB code which includes segmentation and validation. In this paper, we have extracted the basic features of retinal image like optic disc, blood vessels and macula region. The effectiveness of algorithm is validated by comparing the segmented images with corresponding ground truth images. The various evaluation parameters used are Accuracy, Sensitivity, Precision, F-measure, MCC, Dice index, Jaccard index and Specificity.

**Index Terms**— Adaptive thresholding, Fundus Autofluorescence Imaging, Homomorphic filtering, Segmentation.

## 1 INTRODUCTION

RETINA is the inner most layer of eye which contains photoreceptors responsible for vision. Any disease affecting this region will lead to severe problems in vision if not detected in its early stage [1]. Imaging techniques [2] [3] are widely used for the identification of retinal diseases. Optic disc [4] [5], blood vessels [6] [7] and macula [8] are vital parts of retina as many diseases like Glaucoma and diabetic retinopathy [9] are identified by the modification of their size and shape. Optic disc is the origin of blood vessels which nourishes the retinal cells and Macula, located at the center of retina contains a large number of cone cells responsible for colour vision.

Blue wave fundus autofluorescence imaging is a non-invasive technique [10] in which retinal cells are illuminated by blue light of wavelength 488nm [11] and the light gets reflected from cells having fluorophores only. The fluorophores are present in retina except the optic disc and macular region. So, the region of optic disc and blood vessels appears to be darker than other regions. Image processing techniques help the ophthalmologist to identify even the minute changes in this area. Segmentation [12] of these regions is the preliminary task in image processing, which separates it from its background. Conventional image processing tools or deep learning methods like CNN [13] [14] can be used for effective segmentation.

The presence of speckle noise and non-uniform illumination [15] are the major problems while dealing with these images. Image enhancement [16] techniques like adaptive histogram equalization and filtering techniques could improve the quality of image before segmentation.

In this paper, we are proposing an algorithm to segment the optic disc, blood vessels and macular region from fundus autofluorescence images using conventional image processing tools in MATLAB software.

## 2 MEDICAL IMAGES

Blue wave fundus autofluorescence images of healthy retina are selected for implementing segmentation algorithm. 30 FAF retinal images of 15 patients were collected from a local eye hospital. The images were captured with a Heidelberg Engineering instrument which uses blue laser light at 488nm for illumination. The fluorophores present in retina could reflect this light and the image is captured. All images were in JPEG format of the size 1536 X 1636 pixels centered on the macular region with a resolution of 96dpi.

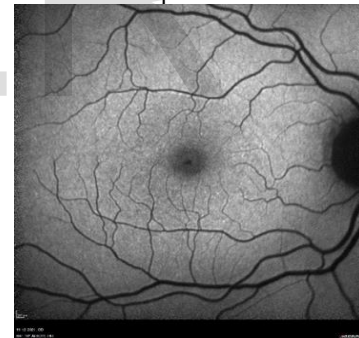


Fig. 1. FAF retinal image of a healthy eye

Both the optic disc along with blood vessels and the macula region do not have any fluorophores which could reflect light. So, they appear to be darker (hypo-fluorescent areas) [17] in blue wave fundus autofluorescence imaging than their background.

## 3 METHODOLOGIES

The segmentation of optic disc, blood vessels and macular region is done by developing and executing a Matlab code, which could precisely identify and extract the features separately. The major steps in the algorithm include pre-processing, segmentation and final processing. The segmentation algorithm for the optic disc, macular region and blood vessels is discussed in this session.

### 3.1 Segmentation of Optic disc and Macula

The various steps in the segmentation algorithm are as shown in the flow chart below.

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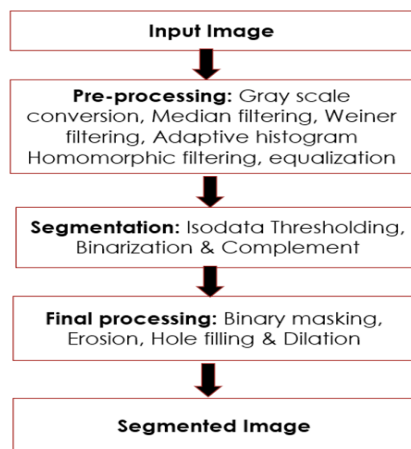


Fig. 2. Shows the flowchart of algorithm for segmentation of macula and optic disc

### 3.1.1 Pre-Processing:

The image processing steps commence with pre-processing techniques like resizing of image for reducing computing complexity. The image size is reduced to 584 x 565 pixels. This true colour image is converted to gray scale value by a weighted sum of the red, green and blue components of each pixel.

$$\text{Gray image} = 0.2989 * R + 0.5870 * G + 0.1140 * B.$$

The effect of speckle noise is reduced by median filtering of image. Further, gaussian noise reduction is achieved by weiner filter which adaptively filters neighbourhood pixels of size 3x3 by estimation of the local image mean and standard deviation. Filtered pixel value is estimated as

$$b(n1, n2) = \mu + \frac{\sigma^2 - v^2}{\sigma^2} (a(n1, n2) - \mu)$$

where  $\mu$  is the local mean,  $\sigma$  is the local standard deviation and  $v$  is the noise variance.

Adaptive histogram equalization technique is used to enhance the contrast of image by dividing the image into tiles (256 tiles) and contrast transformation function is calculated for each tile separately and bilinear interpolation is applied on neighbouring tiles with histogram limited to 128 levels.

The non-linear illumination in image is eliminated by homomorphic filtering. In this, the illumination and reflectance part of an image is separated by applying logarithmic function over the image.  $\ln(m(x, y)) = \ln(i(x, y)) + \ln(r(x, y))$ . The resultant image is then transformed to frequency domain by applying fourier transform and further filtered using a high pass filter to enhance reflectance part (high frequency component) and to get an even illumination over the image.

$$F(\ln(m(x, y))) = F(\ln(i(x, y))) + F(\ln(r(x, y))).$$

$N(u, v) = H(u, v) \cdot M(u, v)$  where  $M$  represents the fourier transform of image,  $H$  is the high pass filter and  $N$  is the filtered image in frequency domain. Image is further transformed to spatial domain through inverse fourier transform followed by application of exponential function. New Image  $f(x, y) = \exp\{\text{inv } F(N(u, v))\}$ .

### 3.1.2 Segmentation:

It is the process of feature extraction from image. This done by binary thresholding method. All the pixel values in the image above a threshold value will be assigned with 1 and rest of the pixels with 0. The threshold value for gray scale image is determined by 'isodata' method in which threshold value is automatically determined from the input image. At first, it assumes a random threshold value and divides the histogram into two classes. Then the average intensity values of these two classes were found and the new threshold value is the average of these two. The computation continues till a convergence is reached.

$$\text{New image } W(x, y) = \begin{cases} 1; & \text{if } f(x, y) > \text{threshold value} \\ 0; & \text{if } f(x, y) < \text{threshold value} \end{cases}$$

To highlight the region of interest, complement of the image is taken.  $C(x, y) = 1 - W(x, y)$ .

### 3.1.3 Final Processing:

It includes the hole filling, binary erosion, masking and dilation process. Hole filling is done to fill background pixels inside closed boundaries to get a uniform region in the binary image. Thin lines in the segmented image are removed by binary erosion using a structural element 'disk'. The unwanted pixels are removed by erosion using the structuring element 'disk'.  $A \ominus B = \{z \mid (Bz \subseteq A)\}$  where  $A$  is the binary image and  $B$  is the structural element. A binary masking method is used to separate the macular region and optic disc region. The final segmented image is obtained by dilation of the binary image using a structural element 'disk', which restores the pixels lost during erosion.

## 3.2 Segmentation of Blood Vessels

The various steps in segmentation of blood vessels are as shown in the flow chart below.

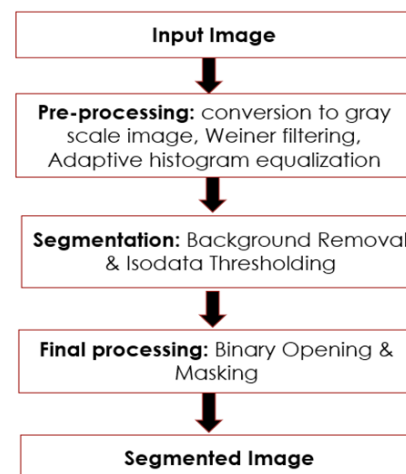


Fig. 3. Shows the flowchart of algorithm for segmentation of blood vessels

Blood vessels spread across the walls of retina and appear to be dark thin network of lines in the image due to the absence of fluorophores.

### 3.2.1 Pre-Processing:

Image is resized to 584 x 565 pixels for reducing computing complexity. True colour image is converted to gray scale im-

age using principal component analysis method. In this, R, G, B components of image is transformed to a Lab colour space where 'L' represents the luminance, 'a' represents red/green tones and 'b' represents yellow/blue tones in the image. Principal components of the images are extracted and gray scale images are generated. Further, gaussian noise reduction is achieved by weiner filter which adaptively filters neighbourhood pixels of size 3x3 by estimation of the local image mean and standard deviation. Filtered pixel value is estimated as

$$b(n1, n2) = \mu + \frac{\sigma^2 - v^2}{\sigma^2} (a(n1, n2) - \mu)$$

where  $\mu$  is the local mean,  $\sigma$  is the local standard deviation and  $v$  is the noise variance.

Adaptive histogram equalization technique is used to enhance the contrast of image by dividing the image into tiles (256 tiles) and contrast transformation function is calculated for each tile separately and bilinear interpolation is applied on neighbouring tiles with histogram limited to 128 levels.

### 3.2.2 Segmentation:

The major steps in this process are the elimination of background of image and thresholding to create a binary image. The elimination of background is achieved by subtracting the image from its own blurred image. Blurring of image is achieved by average filtering with a mask of size 9 x 9 pixels where each pixel in the image is replaced by the average value of 9 x 9 pixels neighbourhood.

Background eliminated image = Blurred image - pre-processed image.

The threshold value for gray scale image is determined by 'isodata' method in which threshold value is automatically determined from the input image. At first, it assumes a random threshold value and divides the histogram into two classes. Then the average intensity values of these two classes were found and the new threshold value is the average of these two. The computation continues till a convergence is reached

$$\text{New image } W(x, y) = \begin{cases} 1; & \text{if } f(x, y) > \text{threshold value} \\ 0; & \text{if } f(x, y) < \text{threshold value} \end{cases}$$

### 3.2.3 Final Processing:

It includes binary opening process followed by masking. Binary opening is applied to remove less connected pixels. All areas less than 100 pixels were removed from the segmented image. Finally, the label at the bottom of the image is removed by binary masking operation.

## 4 RESULTS AND DISCUSSION

The algorithms for segmentation of macular region, optic disc and blood vessels were tested on 30 clinical images and results are validated against ground truth images approved by expert ophthalmologist. Output images shows that the algorithm is an effective solution to segmentation of features in retina.

### 4.1 Segmentation of Macular Region

The following figures shows the major steps involved in the segmentation of macula from blue wave fundus autofluorescence image.

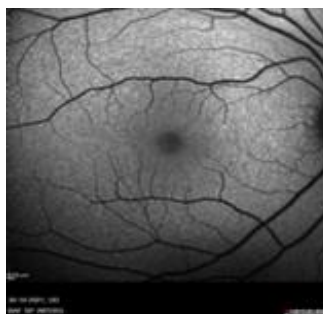


Fig. 4.1. Original Image

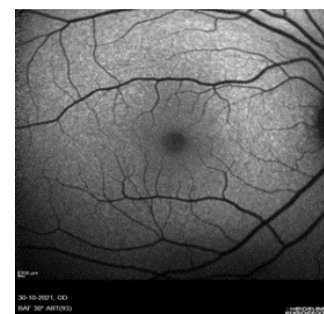


Fig. 4.2. Gray scale Image

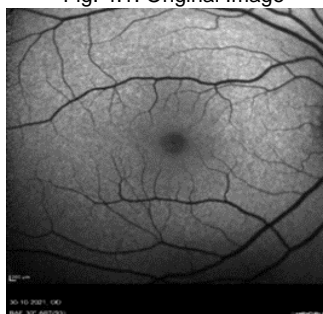


Fig. 4.3. Median filtered Image

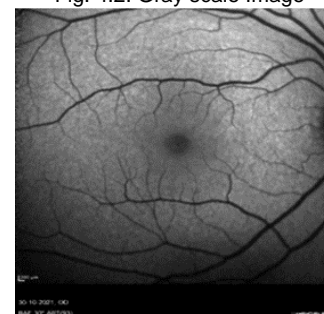


Fig. 4.4. Weiner filtered Image

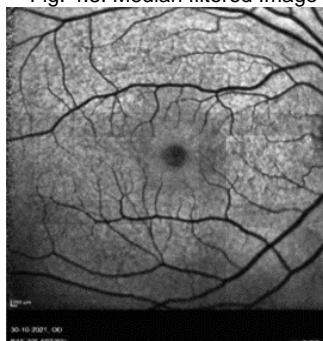


Fig. 4.5. Contrast enhanced Image



Fig. 4.6. Homomorphic filtered

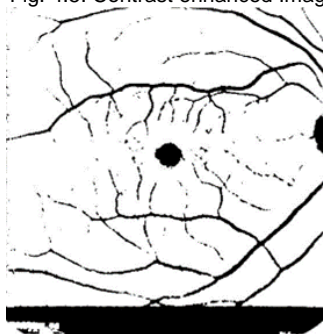


Fig. 4.7. Binary thresholded Image

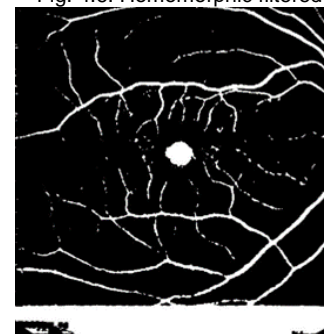


Fig. 4.8. Complement of Image

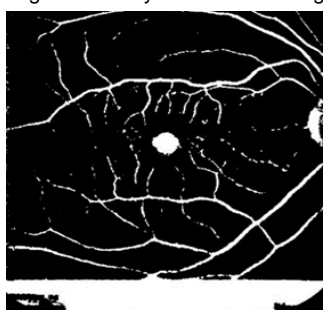


Fig. 4.9. Hole filled Image



Fig. 4.10. Eroded Image

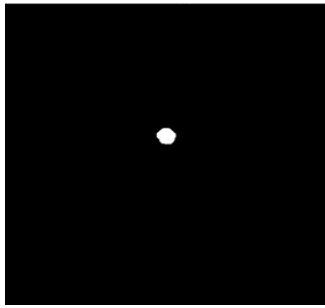


Fig. 4.11. Segmented Macular Region after masking

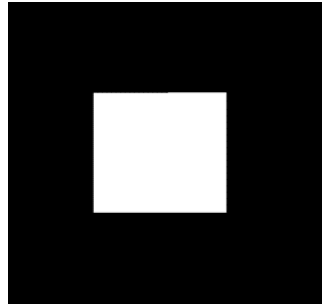


Fig. 4.12. Binary mask

Fig. 4 Shows the images during the different steps in segmentation of macula.

#### 4.2 Segmentation of Optic Disc

The following figures shows the major steps involved in the segmentation of optic disc from blue wave fundus autofluorescence image.

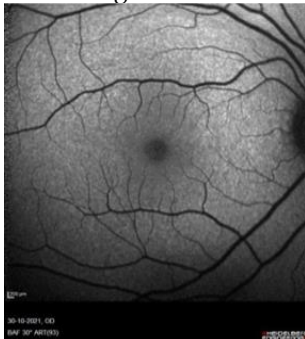


Fig. 5.1. Original Image



Fig. 5.2. Gray scale Image



Fig. 5.3. Median filtered



Fig. 5.4. Weiner filtered



Fig. 5.5. Contrast enhanced



Fig. 5.6. Homomorphic filtered

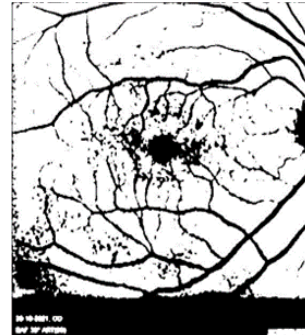


Fig. 5.7. Binary thresholded



Fig. 5.8. Complement of image



Fig. 5.9. Masked image



Fig. 5.10. Eroded image



Fig. 5.11. Segmented Optic disc



Fig. 5.12. Binary mask

Fig. 5 Shows the images during the different steps in segmentation of optic disc

#### 4.3 Segmentation of Blood Vessels

The following figures shows the segmentation of blood vessels from blue wave fundus autofluorescence image.

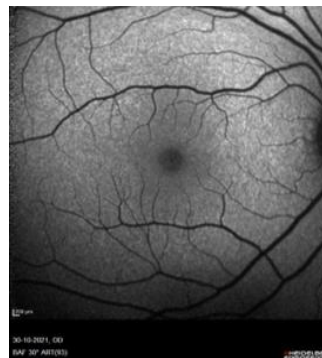


Fig. 6.1. Original Image

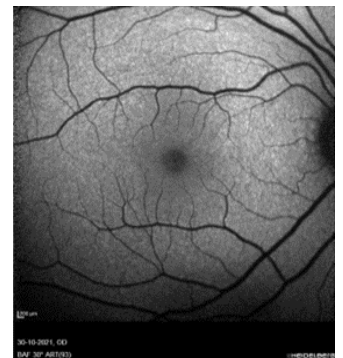


Fig. 6.2. Gray scaled Image



Fig. 6.3. Weiner filtered



Fig. 6.4. Blurred Image

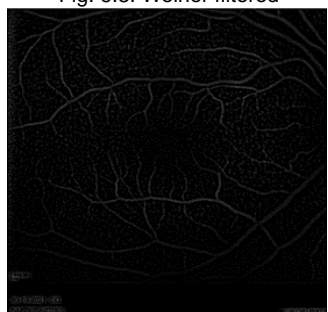


Fig. 6.5. Back ground eliminated Image

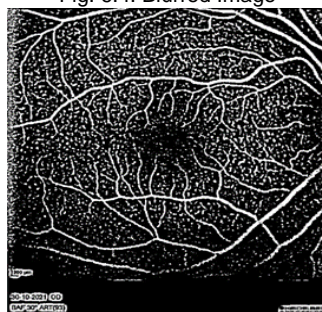


Fig. 6.6. Binary thresholded image



Fig. 6.7. Binary Opened Image

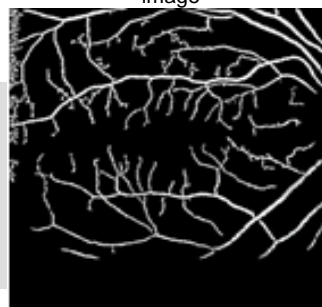


Fig. 6.8. Blood Vessels

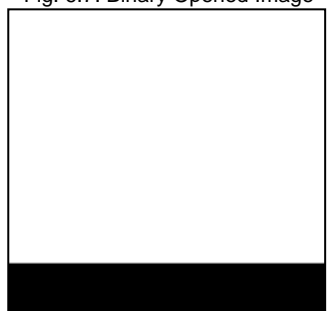


Fig. 6.9. Binary Mask

#### 4.4 Validation of Segmentation

The effectiveness of algorithm for segmentation can be validated by comparing the segmented binary images against its ground truth binary images and calculating the validation scores of the standard parameters such as Accuracy, Sensitivity, Precision, F-measure, MCC (Matthews Correlation Coefficient), Dice index, Jaccard index and Specificity.

The figure below shows the sample segmented images and its corresponding ground truth images.

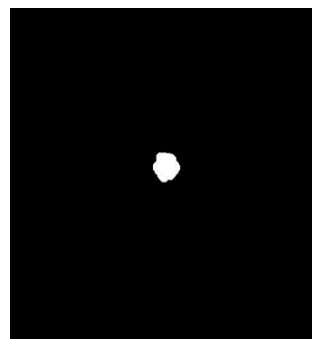


Fig. 7.1 segmented macular region

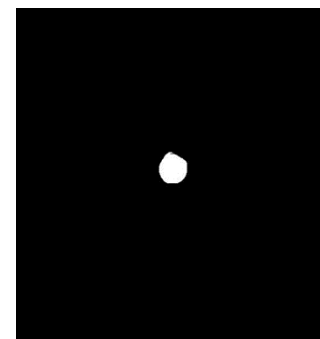


Fig. 7.2 ground truth macular region



Fig. 7.3 segmented optic disc



Fig. 7.4 ground truth optic disc



Fig. 7.5 segmented blood vessels



Fig. 7.6 ground truth blood vessels

Fig. 7 shows sample segmented images and its corresponding ground truth images

The computation of validation is done based on the following relations,

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FN + FP}$$

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

$$\text{Precision} = \frac{TP}{TP + FP}$$

$$F - \text{measure} = \frac{2 * TP}{(2 * TP + FP + FN)}$$

$$MCC = \frac{TP * TN - FP * FN}{\text{sqrt}((TP + FP) * (TP + FN) * (TN + FP) * (TN + FN))}$$

$$\text{Dice} = \frac{2 * TP}{(2 * TP + FP + FN)}$$

$$\text{Jaccard} = \frac{\text{Dice}}{(2 - \text{Dice})}$$

$$\text{Specificity} = \frac{TN}{(TN + FP)}$$

The following table shows the average values obtained as val-

validation scores against 30 clinical images.

Table.1

Average Validation scores in segmentation

Validation Parameter	Macular Region	Optic Disc	Blood Vessels
Accuracy	0.9982	0.9938	0.9352
Sensitivity	0.7883	0.8091	0.6347
Precision	0.8857	0.7918	0.3810
F-measure	0.8151	0.7685	0.4678
MCC	0.8250	0.7813	0.4567
Dice index	0.8151	0.7685	0.4678
Jaccard index	0.6950	0.6468	0.3083
Specificity	0.9993	0.9966	0.9499

The validation scores indicate that the algorithm could effectively segment the retinal images.

## 5 CONCLUSION

Segmentation is the preliminary process in image processing which helps in early diagnosis of eye diseases. An algorithm for the segmentation of optic disc, blood vessels and macular region is implemented using conventional image processing tools in MATLAB. This algorithm is tested on 30 FAF healthy retinal images of 15 patients and its validation shows a high degree of accuracy and appreciable scores for other parameters.

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