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IMAGE ANALYSIS FOR AUTOMATIC ENUMERATION OF RBC INFECTED WITH PLASMODIUM PARASITES-IMPLICATIONS FOR MALARIA DIAGNOSIS

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ABSTRACT. Plasmodium parasites can cause human to get malaria. Malaria is a common infectious disease and a worldwide problem concerning public health, particularly in Africa and south Asia. Manual microscopy for malaria diagnosis is a time consuming process and relies on the expertise of the technician. The main aim of the proposed method is to develop a decision support system for diagnosis based on enumeration of cells infected with plasmodium parasites in microscopic blood images by using modified iterative thresholding and connected component labeling. We have focused on maintain consistency of illumination that appear frequently in these images through color normalization. The proposed enumeration system is evaluated using an image dataset consisting of over two-hundred microscopic blood images. It is demonstrated that the proposed method is capable of enumeration of infected cells accurately with a highly positive correlation with manual enumeration.

1. INTRODUCTION

Malaria is a critical healthcare problem worldwide. According to the world malaria report of 2019 published by WHO, an estimated worldwide 228 million cases and 40,500 deaths from malaria in which 67 % of deaths are children aged under five years [1]. Malaria infection vanishes when either the patient dies or

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the parasite is defeated by the immune system. The conventional method of diagnosing malaria depends on the microscopic examination of Giemsa-stained blood smears and this technique is considered the gold standard. Approximately, a total of 197 million patients worldwide are tested for malaria by microscopic examination. However, the manual method of diagnosis is tedious, requires expert technicians, and is prone to human erroneous. Hence, it affected the accuracy of the diagnosis (increasing false diagnosis) by pathologists [2]. Considering these problems, there is need for an automated system for diagnosis of malaria. Hence, we propose a automatic enumeration of infected parasites to assist diagnosis. In the rest of the article: the Preliminaries, Methodology, Experimental Results and Conclusions are presented in sections 2 to section 5, respectively.

2. PRELIMINARIES

There are several techniques exists for diagnosis of malaria through classification, segmentation, detection and malaria count through microscopic imaging [2]. A contrast enhancement microscopic images as a preprocessing step proposed in [3]. A novel algorithm for morphological filtering of microscopic images for segmentation proposed in [4]. The malaria infected erythrocytes segmentation based on Fuzzy c-means clustering proposed in [5]. The gamma correction and adaptive median filter used for preprocessing techniques. In [6], detecting and identification of parasites in microscopic blood images is proposed. Besides, the method identifies life-cycle stages and the infecting species.The preprocessing step applied for illumination correction. In [7], marker-controlled watershed transformation used for segmentation of malaria infected cells. Otsu method used for binary mask of infected cells [8]. The adaptive thresholding method used for segmentation of infected cells and closing operation applied as a post processing stage with an elliptical structuring element. The method make classification of four species of malaria parasites and three life-cycle stages for species. The SE and SP of the method yields 74-96% and 93-99%, respectively [9]. The adaptive thresholding method used for detecting malaria infected cells in [10]. The erosion and dilation operations used for removing unwanted pixels in segmented binary image. The accuracy for thin and thick films of the proposed the method are 97.8 and 89.8, respectively.

In [11], quantification of erythrocytes in images for computer supported diagnosis system proposed and the accuracy of the method is 98.02%. Enumeration of infected cells based on learning by sampling proposed in [12]. In [13], low contrast image classification measure for malaria images proposed. This measure classifies low and normal contrast blood images. Confusion matrix used for classification performance. In [14], Image analysis and machine learning methods used to quantify infected cells for diagnosis. The various segmentation and classification of malaria parasites through microscopic imaging discussed in [2,14,15]. The proposed method enumerates malaria infected cells for diagnosis and its works well for both infected and normal images. In infected images, the enumeration is more than or equal to one where as for normal images the enumeration is zero.

3. METHODOLOGY

The block diagram of proposed method for automatic enumeration of malaria parasites for diagnosis as shown in Fig. 1.

3.1. **Normalization.** Normalization is the process that changes the range of pixel intensity values. Due to various experimental conditions, the colors are not equally distributed in microscopic images. So we normalize the color consistency. Also it is very difficult for segmentation for low contrast images. Hence it is necessary to normalize the color channels for RGB images for accurate enumeration. Suppose, an RGB image of size m$\times$n is f(x,y) where x and y denote the indices of the pixel position. We denote Red, Blue and Green components are f_R (x,y), f_G (x,y) and f_B (x,y), respectively.

The average value Red, Green and Blue channels are calculated by using the following equation. Where I$\in$R,G,B

$$I_{avg} = \frac{1}{mn} \sum_{x=0}^{m-1} \sum_{y=0}^{n-1} f_I(x, y).$$

The inverse of the average values of the three channels are given by,

$$IN_I = (I_{avg}^{-1}).$$

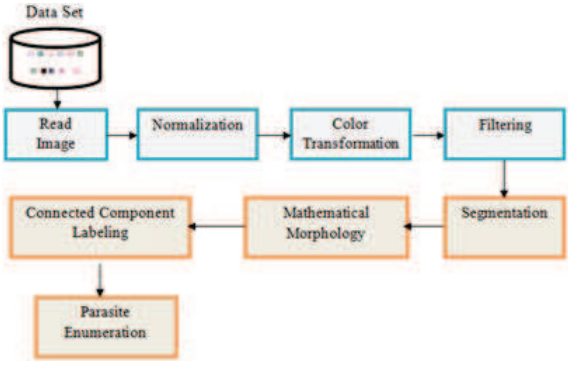

FIGURE 1. Block diagram of the proposed method

The scaling factors are computed for each channel by using the following equations.

$$SR = \frac{IN(I)}{max(IR, IG, IB)}.$$

The color corrected image is formed with f_RCORR ,f_GCORR and f_BCORR and are computed by using the following equation.

$$f_{RCORR}, f_{GCORR}, f_{BCORR} = (SR) * f_R(x,y), (SG) * f_G(x,y), (SB) * f_B(x,y).$$

Often, the RGB image f(x,y) has sufficient intensity range for all the channels which assist to improve the enumeration results performance to assist diagnosis. The original image and normalized image along with histograms of original and resultant image is shown in Fig. 2. Color space conversion is a pre-process used to transform 24-bit RGB image g(x,y) to 8-bit gray level image [15,16]. The adaptive median filter is used for noise reduction [5].

3.2. **Parasite Extraction.** In medical imaging, segmentation plays a vital role as it extracts the region of interest (ROI) for computer aided diagnosis. Due to heterogeneity of microscopic blood images, it is difficult to fix threshold value directly or automatically [5]. Therefore, the optimal threshold value for parasite extraction is done by using two stages processing of image. In first-stage, some of the cells/regions are neglected which are not infected by plasmodium parasites for further step by pre-optimal threshold value with help of Otsu thresholding [17]. The initial stage (stage-one) image is considered for calculating optimal threshold value and the pseudo-code for finding optimal threshold value for extracting malaria infected cells is shown in Algorithm.1.

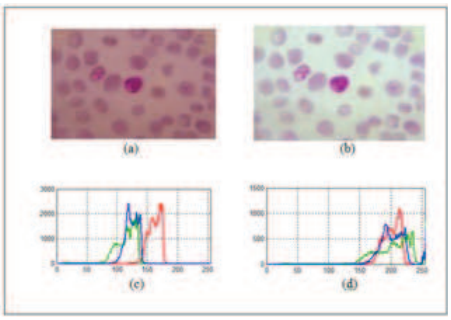

FIGURE 2. Normalization for microscopic blood RGB Images: (a) original image, (b) image after normalization, (c) histogram of original one, and (d) histogram of normalized image

Algorithm 1.Pseudo-code for optimal threshold selection.

F(x,y): (STAGE-ONE THRESHOLDED IMAGE OF SIZE mxn)
T_{INIT}: (INITIAL THRESHOLD VALUE)
T_{NEW}: (UPDATED THRESHOLD VALUE)
F_{MAX} : (MAXIMUM GRAY VALUE OF AN IMAGE F)
F_{MIN} : (MINIMUM GRAY VALUE OF AN IMAGE F)
Initialize:

- $T_{INIT} \leftarrow \text{Mean}(F_{MAX}, F_{MIN})$
- $\forall\ S_i, N_i \leftarrow 0$ for $i = 1,2.$
- x=y=0

Run:

Repeat
while (x<=m-1) do
while(y<=n-1) do
if $(F(x,y) \geq T_{INT})$ then

$$P \leftarrow \left\lfloor S_1 + \sum_{x=0}^{m-1}\sum_{y=0}^{n-1} F(x,y) \right\rfloor$$

N_1+=1;
Else

$$Q \leftarrow \left\lfloor S_2 + \sum_{x=0}^{m-1}\sum_{y=0}^{n-1} F(x,y) \right\rfloor$$

N_2+=1;
end if
y + =1
end while
x+=1
end while

$$F_{NEW\_MIN} \leftarrow \frac{P}{N_1}$$

$$F_{NEW\_MAX} \leftarrow \frac{Q}{N_2}$$

T_{NEW}←Mean(F_{NEW_MIN} ,F_{NEW_MAX})
$D \leftarrow ABS(T_{INT}, T_{NEW})$
$T_{INT} \leftarrow T_{NEW}$
Until D converge.

FIGURE 3

3.3. **Mathematical morphology.** In order to make the segmented object look better, the objects in the processed image can be smoothed by eroding the image with 3×3 structuring element. It removes outer layer of pixels yields more

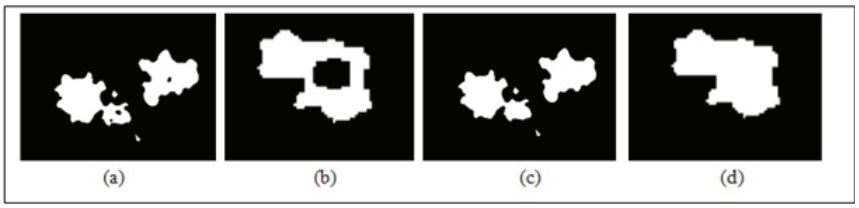

FIGURE 4. Hole filling illustrations: (a) and (b) are images with holes,(c) and (d) are images with hole filling, respectively

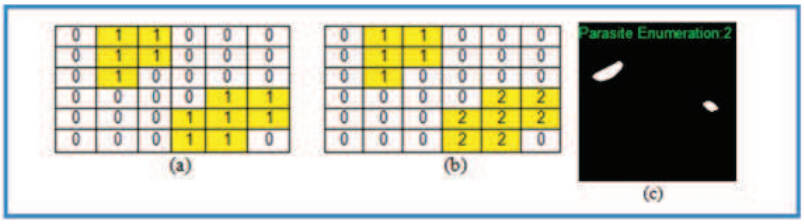

FIGURE 5. Illustration of CCL algorithm results

accurate enumeration results. If a black pixel has a white neighbor, then all the pixels are made white [15,17]. In order to enumerate malaria parasite more accurately, it is necessary to fill holes inside the parasites infected regions [5]. The results after applying hole filing process to few sample images are shown in Fig. 4.

3.4. **Connected Component Labeling.** The enumeration of malaria parasites carried out by using connected component labeling (CCL) algorithm. In CCL, the connected components are identified and assigned label for each connected component for enumeration. All pixels of the each connected component have similar values. Pixel connectivity is used to identify which pixels are connected to other pixels in the surrounding neighborhoods [17]. We have chosen 8-connected neighborhood for accurate results [5]. The results obtained by applying CCL algorithm to the sample binary matrix and binary images are shown in Fig. 5.

3.5. **Experimental Results.** The malaria infected cells enumeration in microscopic images results are shown in Fig. 6. For experimental processing MATLAB programming software is used. The proposed method tested for infected and

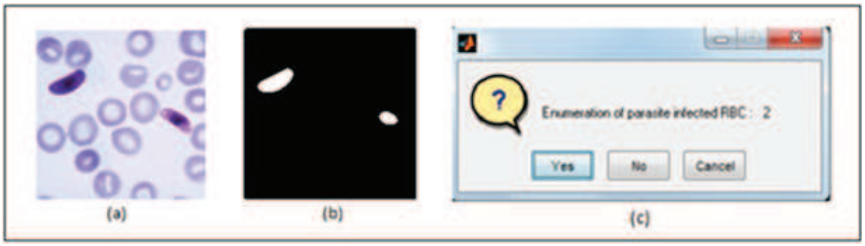

FIGURE 6. Enumeration results by the proposed method

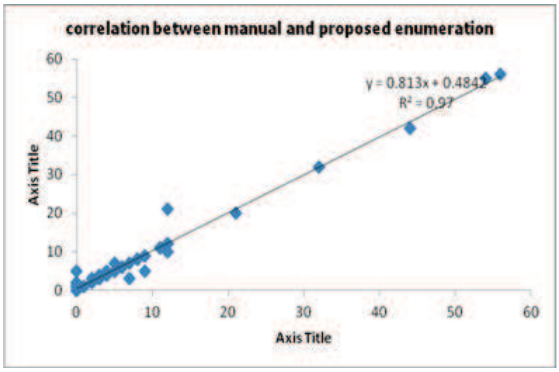

FIGURE 7. Correlation between manual and proposed enumeration

normal microscopic images and hence leads to more accurate results. The correlation coefficient between proposed one and manual count is highly correlated and shown in Fig. 7. If the enumeration is non-zero then result of diagnosis of malaria is positive otherwise negative.

4. CONCLUSION

In this paper, we have proposed a method for enumeration of malaria infected cells in microscopic blood images for assisting diagnosis. The RGB image is converted in to into grayscale image for single channel processing. The pixel intensity values are normalized due to heterogeneity, illumination effects etc., for accurate enumeration. The infected parasites are segmented by the using the proposed optimal value in second stage of image thresholding. The proposed enumeration results are highly correlated with manual count of infected cells.

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