



Automated Detection of Morphological Implications on RBC Consequent to Infections with Human *Plasmodium vivax* and *Plasmodium falciparum*

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Abstract— Morphological changes during infection with *Plasmodium vivax* and *Plasmodium falciparum*. The most used test is the blood smear on microslide that is stained to show the parasites inside RBC. The current diagnostic methods can help in correct diagnosis but cannot give any measurable changes that occur in cytoplasm induced by *Plasmodium vivax* and *Plasmodium falciparum* on the host erythrocytes (RBC). In this proposed technique, the microscopic images of the infected erythrocytes are obtained from the electron microscope. The image is preprocessed to remove noise and segmentation is applied to detect the edges. Then contour extraction is done to extract the features like perimeter, area, form factor etc. Finally the morphological changes of the infected RBC's are compared with that of normal RBC's and the level of infections are determined. The proposed method provides a solution for statistical identification of infected RBC's by determining the changes in the erythrocytes and the severity of the disease with the level of infestation. In addition to that zooming operations and histogram generation can be performed by the system for any microscopic images given as input.

Keywords— *Plasmodium falciparum*, *Plasmodium vivax*, Zooming, Aggregates, Erythrocytes

I. INTRODUCTION

Malaria is a serious infectious disease caused by a peripheral blood parasite of the genus *Plasmodium*. According to the World Health Organization, it causes more than 1 million deaths arising from approximately 300 to 500 million infections every year. Even today malaria continues to remain one of the most prevalent infectious diseases in the world, with the African continent being the largest malaria stronghold on the globe. Malaria is an acute infectious disease caused by malarial plasmodia transmitted by mosquitoes of the genus *Anopheles*.

In 1897 Ronald Ross working as a military physician in India, made another extremely important discovery, proving experimentally that mosquitoes serve as vectors of human malaria. Although there are newer techniques, manual microscopy examination of blood smears (invented in the late 19th century), is currently "the gold standard" for malaria diagnosis. Diagnosis using a microscope requires special training and considerable expertise. It was shown in several studies that manual microscopy is not a reliable screening method when performed by nonexperts due to lack of training especially in the rural areas where malaria is endemic. *Plasmodium vivax* occurs throughout most of the temperate zones and also in large areas of tropics. It is much less common in tropical Africa, especially so in west Africa. It causes so called benign tertian malaria with frequent relapses, the pattern of which varies, in relation to various strains of *P. vivax*. [1].

Sporozoites of *P. vivax* differentiate, after invading the liver. [10] During the erythrocytic development of *P. vivax*

all blood forms can be found in the circulation and most stages are larger than in other species of human plasmodia. [5] The young trophozoites or ring grows rapidly and soon exhibits the characteristic malaria pigment. *Plasmodium falciparum* is the most highly pathogenic, as is indicated by the name malignant often applied to the type of malaria associated with it. The young ring forms of *P. falciparum*, as usually seen in the peripheral blood, are very small, measuring about one-sixth of the diameter of Red blood cell. Later in the attack the ring forms of *P. falciparum* may be considerably larger, measuring one-quarter and sometimes nearly one-half the diameter of red blood cells. [2] The distribution of the parasites in organs and tissues of the human body varies in different cases, thus accounting for the diversity of clinical manifestations observed in *falciparum* malaria. [8] Most of the severe and fatal cases are due to blocking of the capillaries by clumps of Red blood cells harbouring developing parasites, enormous numbers of which can be seen in smears and sections of postmortem material. [11] The development of the gametocytes takes place in the inner organs, but sometimes young forms are seen in the blood. [3] The young gametocytes tend to be elongated, becoming spindle-shaped or elliptical as they grow. Finally they assume the characteristic curved shape of the mature gametocytes.

II. MATERIAL AND METHODS

The positive blood films on microslides were obtained from the malaria clinic at the Zonal Entomological Team at Vellore. Low, medium and high parasitemia blood samples were thus determined and stained using Leishman stain as

per guidelines of the National Vector Borne Diseases Control Programme.

Leica DM 2000 Microscope for Research Laboratories was used in the study during slides examination and images capture.[9] The outstanding brilliance and clarity of the images, the ability to use the full range of contrast and light microscopy techniques to reveal the most delicate structures. Leica's digital imaging program is optimized for the Leica DM Series with image analysis and management capabilities, and includes digital cameras, image processing workstations, and sophisticated software for image organization and archiving. The entire shape analysis was carried out by employing the GUI using MATLAB-R2007b software supported with Windows 2000/XP operating system.

The Leica Microscope has the following properties:

- A. 30 W illuminations,
- B. Bright field, dark field,
- C. Phase contrast,
- D. Polarization contrast,
- E. Differential Interference Contrast (DIC),
- F. Fluorescence microscopy,
- G. Standard coarse and fine focus with optional super-fine focus,
- H. Integrated thermal compensation eliminates focal
- I. drift, which stabilizes stage height during long-term observation and ensures consistent image sharpness,
- J. Use with objectives down to 2.5x.

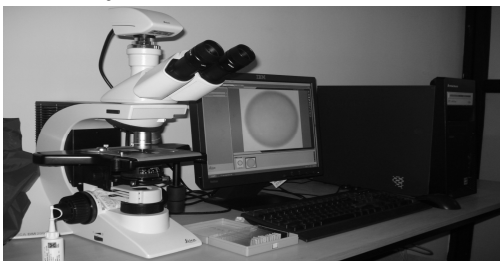


Figure1. Leica Microscope

III. PROPOSED SCHEME

A. Analysis of Morphological Implications in RBC Due to Infections:

The given image is displayed on the computer monitor. IMREAD reads image from graphics file. The RGB image on the screen is converted to a gray scale image. `rgb2gray` convert RGB images to gray scale by eliminating the hue and saturation information while retaining the luminance. The gray scale image obtained above procedure has a noisy pattern of isolated intensity in the background. In order to avoid patches the image was filtered by a median filter.

For this the pixel gray levels were compared with a selected threshold value and were classified depending on whether the pixel value was greater or lesser than the threshold level.[4] By this procedure a binary image was obtained. To achieve the edge enhancement the Sobel operator was used. `EDGE` takes intensity or a binary image *I* as its input, and returns a binary image *BW* of the same size as *I*, with 1's where the function finds edges in *I* and 0's elsewhere. The Sobel method finds edges using the Sobel approximation to the derivative. It returns edges at those points where the gradient of *I* is maximum.[7] This was achieved by successive deletion of the outer-most layer of

the image until a connected unit pixel width framework or skeleton remained. By counting the number of pixels along the perimeter and then multiplying with pixel length, the perimeter (*P*) of the same cell was determined. For calculation of area, the contour of the cell was filled up with pixels. By counting the number of these and multiplying with pixel area, the area (*A*) of the cell was determined. Based on above area and perimeter, the form factor ($FF = P^2/4\pi A$) was calculated as shown in fig.2. This parameter is the measure of roundness of the cell and its variation indicates the deviation of the shape in the image from that of a disc (FF for disc = 1). Zooming operation is performed using pixel replication techniques. Similarly using background subtraction the infected area is extracted and its area, perimeter, and form factor are found as shown in fig.3.

B. Comparison Between Normal, low, Medium, High Parasitemia:

Using the values that is obtained from the above section a comparison bar chart is computed by giving those values as input. Each bar has different width and color as shown in Fig.2

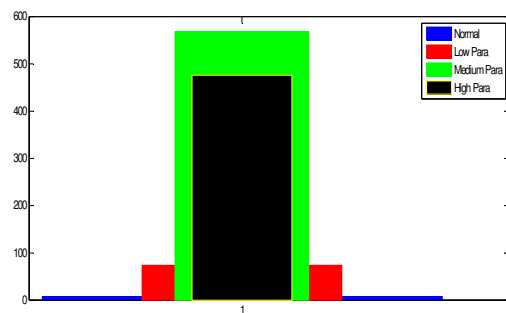


Figure.:2Comparison between normal low,medium,high Parasitemia

C. Zooming Operation and Histogram Generation:

One of the valuable techniques used in the microscopy technology is zooming of the slides. In this system, that technique is made available using the concept of pixel replication.[6] The zooming size is got as input from the user. Based on that zooming size or factor the pixel is replicated both row wise and column wise. As a result of which we get a zoomed image as in fig 3

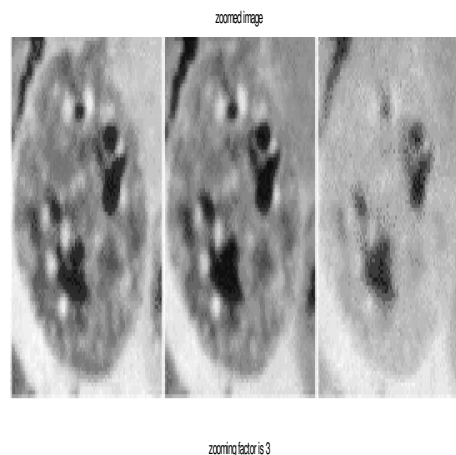


Figure.3 Zoomed image with zooming factor = 2

For Histogram generation HSV colour space is used. The histogram of the image gives the frequency of occurrence of the gray level. It gives the number of times a particular gray level occurs in the image as in fig 4.

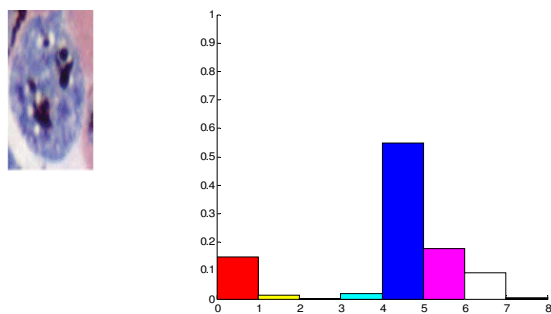


Figure.4 Histogram for a colour image

D. Detection of levels of Infection:

Total number of RBC's, number of infected RBC's, Parasitemia percentage in an image can be detected. The border of the image is cropped and the green channel is extracted. Then the image is converted to double format. Then it is converted to binary image. Then Canny's edge detection is performed.[7] Then image is labeled. Terminal end points of edge contour are detected and endpoints are linked to form the closed set of contours. Then image morphology is done to find the region of interest. The unfilled contours are filled. Then the smaller regions and elongated objects are removed. Then overlapping objects are segmented and then total number of RBC's is found. Then features of interest with in each object are analyzed and number of infected RBC's is found. Finally parasitemia percentage is detected as in fig 5.

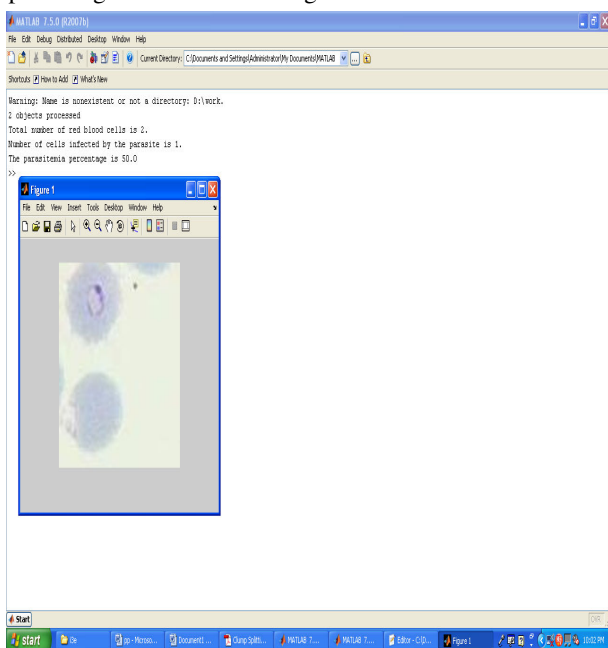


Figure. 5 Detection of number of cells, number of infected cells and parasitemia%

IV. PERFORMANCE EVALUATION

The system is tested with more than 25 images of blood samples of the patients. The morphological implications are analyzed and the result is displayed on the screen which includes the area, perimeter, form factor, infected area with a comparison chart. Using the Zooming operation, zoomed image of varying size has been displayed. Even Histogram has been generated for the colour image. Then total number of RBC's, number of infected RBC's, and parasitemia percentage has been found accurately for more than 25 images. The system proved to be efficient in all the cases and proved to be an important automated detection system.

V. CONCLUSION

The proposed scheme provides a solution for statistical identification of infected RBC's by determining the changes in the erythrocytes due to infections with the parasites. The severity of the disease with the level of infestation for several images has been found to be correct. In addition to that zooming operations and histogram generation present in this scheme are some of the essential features needed for an automated system.

VI. ACKNOWLEDGEMENT

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