AUTOMATIC DIFFERENTIATION BETWEEN RBC AND MALARIAL PARASITES BASED ON MORPHOLOGY WITH FIRST ORDER FEATURES USING IMAGE PROCESSING

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ABSTRACT

Malaria is the most important parasite infection of human and is associated with a huge burden of morbidity and mortality in many parts of tropical world. The world health organization estimates 300-500 million malaria cases and more than 1 million deaths per year. The definitive diagnosis of malaria infection is done by searching for parasites in blood slides (films) through a microscope .However; this is a routine and time consuming task. Besides a recent study on the field shows the agreements rates among the clinical experts for the diagnosis are surprisingly low. Hence, it is very important to produce a common standard tool which is able to perform diagnosis with same ground criteria uniformly everywhere. Techniques have been proposed earlier that makes use of thresholding or morphology or segment an image .Here I have presented a technique that takes benefits of morphological operation and thresholding at appropriate position in the hole process to maximize the productivity of algorithm and differentiate between the simple RBC and malaria parasite. An approach presenting here to detect red blood cells with consecutive classification into parasite infected and uninfected cells for estimation of parasitaemia.

KEYWORDS: *parasites, morphology, segmentation, diagnosis, thresholding*

I. INTRODUCTION

Malaria cannot be passed directly from one human to another. It can be transmitted by a mosquito [2].The incubation period for malaria varies considerably. For the most serious form of malaria, the incubation period is eight to twelve days. In some rare forms of malaria, the incubation period can be as long as ten months [3].

A lot of research has been carried out in automatic processing of infected bloods cells- Jean-Philippe Thiran in his paper [4] described a method for automatic recognition of cancerous tissues from an image of a microscopic section. This automatic approach is based on mathematical morphology. This method pays no special attention to the speed of the algorithms.

An accurate technique for the determination of Parasitaemia has been suggested in Selena W.S. Sio [7]. The algorithm has four stages namely edge detection, edge linking, clump splitting and parasite detection. The value of PPV given by Sio is 28-81% and it takes 30 Seconds to process a single image.

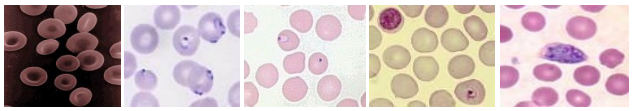
F. Boray Tek [8], transforms the images to match a reference image colour characteristics. The parasite detector utilizes a Bayesian pixel classifier to mark stained pixels. The value of sensitivity given by 74% and value of PPV given by 88%, and pays no special attention to the speed of the algorithms.

The objective of our work is to develop a fully automated image classification system to positively identify malaria parasites present in thin blood smears, and differentiate the species.. The effort of the algorithm is to detect presence of parasite at any stage. So if this algorithm is incorporated in routine tests, the presence of malaria parasite can be detected.

II. MORPHOLOGICAL FEATURES OF RBC AND MALARIA PARASITES

2.1 Morphological features of RBC-RBCs are among the smallest cells normally disc-shaped, soft and flexible, and red in color in the body (the smallest is sperm) and the most numerous type of cell present in the blood.. A typical RBC has a diameter of 7.7 µm (micrometer) and a maximum thickness of roughly 2.6 µm, but the center narrows to about 0.8 µm. The total surface area of the RBC in the blood of a typical adult is roughly 3800 square meters -- 2000 times the total surface area of the body.

2.2 Morphological features of malarial parasites-There are four types of human malaria – **Plasmodium falciparum**, **P. vivax, P. malariae**, and **P. ovale**. **P. falciparum** and **P. vivax** are the most common. P. falciparum is by far the most deadly type of malaria infection



(a) (b) (c) (d) (e)

Figure 1: -(a)Simple RBC(a) Plasmodium Falciparum (b) P.Vivax P.Malariae (d) P.Ovale

Table 1: Morphological features of the host red blood cell by species of Plasmodia in stained thin blood film

	P. Falciparum	***P. Vivax***	***P. Ovale***	***P. Malariae***
Age	Young and old erythrocyte s infected	Young erythrocytes infected	Young erythrocytes infected	Older erythrocyte s infected
Dimension s	Normal	Enlarged	Enlarged, sometimes assuming oval shape	Normal
Color	Normal to dark	Normal to pale	Normal	Normal
Granules	Unusual coarse scattered red stippling in mature trophozoite s or schizonts (Maurer's clefts	Frequent fine red diffuse Stippling in all stages of erythrocytic developmenta l cycle (Schuffner's dots)	Frequent fine red diffuse stippling in all stages of erythrocytic developmenta l cycle (Schuffner's dots, also called James' dots)	None
Granules	Unusual coarse scattered red stippling in mature trophozoite s or schizonts (Maurer's clefts	Frequent fine red diffuse Stippling in all stages of erythrocytic developmenta l cycle (Schuffner's dots	Frequent fine red diffuse stippling in all stages of erythrocytic developmenta l cycle (Schuffner's dots, also called James' dots)	None
Pigment	Dark brown and Usually compact	Golden brown and usually loose	Brown coarse pigment granules	Brown coarse scattered pigment granules
Leucocytes	The presence of malaria pigment in neutrophils and monocytes is a prognostic marker of severe disease			

III. THE STEPS OF ALGORITHM

Visual quantification of parasitemia in thin blood films is a very tedious, subjective and time-consuming task. This selected algorithm presents an original method for enumeration and

classification of erythrocytes in stained thin blood films infected with malarial parasite.
The process is given below.

1. Image Acquisition (Done using high resolution Digital Camera)
2. Image Analysis
3. Image Segmentation
4. Feature Generation
5. Classification of Parasite and result verification

3.1 Image acquisition and database collection

Oil immersion views (10x1000), of Giemsa stained blood films were captured using a binocular microscope mounted with a digital camera. Captured images were 460 pixels X 307 pixels bitmap images.

3.2 Image analysis

Image analysis usually starts with a pre-processing stage, which includes operations such as noise reduction. Canny edge detector, which has become one of the most widely used edge finding algorithms, is found to be ten times slower than this SUSAN approach.

3.2.1 Non linear filtering: SUSAN using for filtering approach

For a real time system using time varying image sequences, speed is an important criterion to be considered. Also there has to be a compromise between maximizing signal extraction and minimizing output noise: the so-called "Uncertainty Principle" of edge detection. I have implemented a new approach to low-level image processing - SUSAN (Smallest Univalue Segment assimilating Nucleus) Principle [10], which performs Edge and Corner Detection and Structure Preserving Noise Reduction.

3.3 Image segmentation:

For the actual recognition stage, segmentation should be done before it to extract out only the part that has useful information. The goal of the segmentation process is to define areas within the image that have some properties that make them homogeneous. After segmentation, the discontinuities in the image correspond to boundaries between regions can be easily established**.**

3.3.1 Segmentation using morphology:

The most commonly used morphological procedure for estimating size distribution of image components is the Granulometry.[9] The size and eccentricity of the erythrocytes are also required for the calculation of some feature values (as these can be indicative of infection). The shape of the objects (circular erythrocytes) is known a priori, but the image must be analyzed to determine the size distribution of objects in the image and to find the average eccentricity of erythrocytes present. Here gray scale granulometries based on opening with disk shape elements are used. Non flat disk shaped structural element are used to enhance the roundness and compactness of the red blood cells and flat disk shaped structural element are used to segment overlapping cells. The object to be segmented differs greatly in contrast from the background image. Changes in contrast can be detected by operators that calculate the gradient of an image. The gradient image can be calculated and a threshold can be calculated and a threshold can be applied to create a binary mask containing the segmented cell. The binary gradient mask is dilated using the vertical structuring element followed by the horizontal structuring element. The cell of interest has been successfully segmented, but it is not the only object that has been found. Any objects that are connected to the border of the image can be removed. The segmented object would be to place an outline around the segmented cell

IV. FEATURE GENERATION AND CLASSIFICATION

4.1 Feature Generation

Two sets of features are used for development. The first set will be based on image characteristics that have been used previously in biological cell classifiers, which include geometric features (shape and size), colour attributes and grey-level textures.

It will be advantageous to apply expert, a priori knowledge to a classification problem. This will be done with the second set of features, where measures of parasite and infected erythrocyte morphology that are commonly used by technicians for manual microscopic diagnosis are used. It's desirable to focus on these features, because it is already known that they are able to differentiate between species of malaria.

4.2 Feature Classification

The final classification of an erythrocyte as infected with malaria or not, and if so, the species of the parasite, falls to the classifier. The classifier is a two-stage tree classifier, with an infection classified as positive or negative at the first node, and the species assigned at the second node.
The design of a tree classifier has the following steps: the design of a tree structure (which has already been assigned), the selection of features to be used at every node, and the choice of decision rule at each node [*12*]. The same type of classifier is used at both nodes.

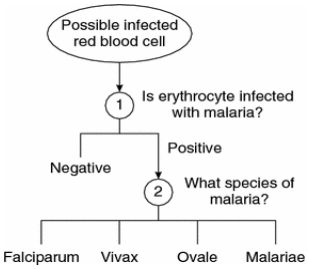


Figure 2: Structure of the tree classifier

The features selected for the first classifier are those that describe the colour and texture of the possible parasites. The features used by microscopists to differentiate malaria species are selected for the second classifier. The training goal is to minimize squared errors, and training is stopped when the error of a validation set increased. This is done to avoid overtraining.

V. RESULTS FOR RBC AND MALARIA PARASITE AFFECTED BLOOD CELL

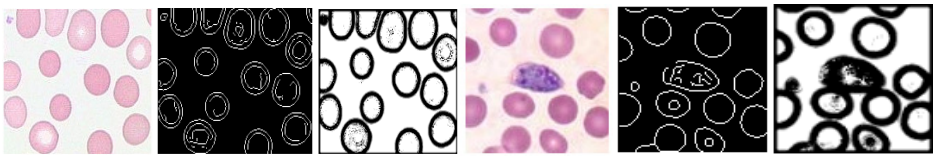
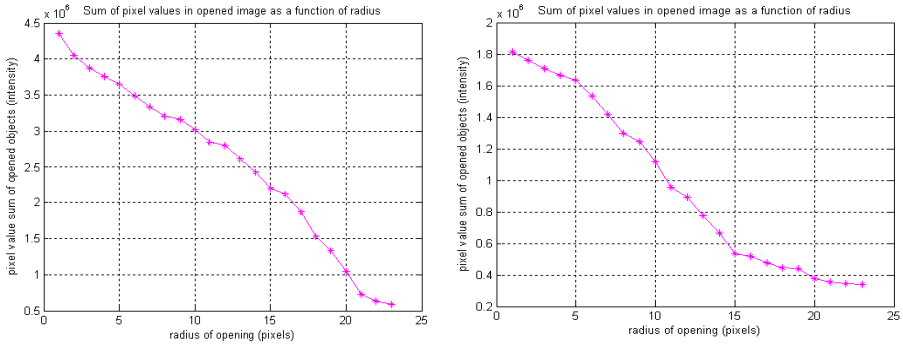


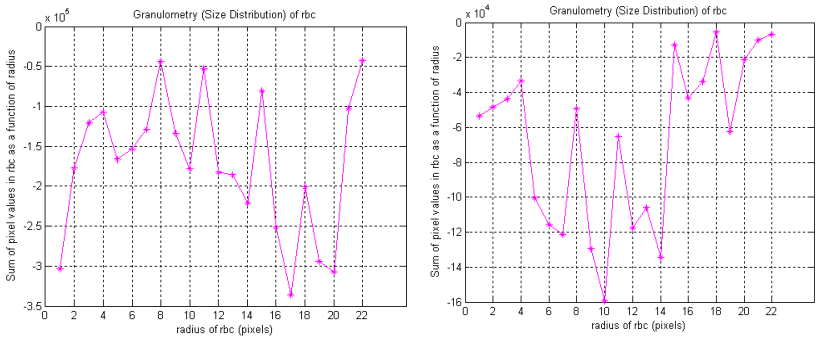
Figure 3:The comparison between output of CANNY and SUSAN algorithm-(a)simple rbc (b)CANNY output of simple RBC(c)Susan output of simple RBC (d)parasite affected blood cell(e)CANNY output of parasite affected blood cell(f)SUSAN output of parasite affected blood cell

We can see in Figure 3,Canny edge detector is the powerful edge detector, but they cannot connect the edges of object, broken edges are mixed with background, very few junction involving more than two edges are correctly connected. Some sharper corners have broken edges. SUSAN provides good detection, good localization, has a single response to a single edge. We can see the edge connectivity at junction is complete, the reported edges lie exactly on the image edges, the edges around and inside the brightness ramp are correctly found and no false edges are reported as faster.



(g) (h)

Figure 4: Graph (g)shows the sum of pixel value in opened image as a function of radius of simple RBC (h) the sum of pixel value in opened image as a function of radius of parasite affected blood cell

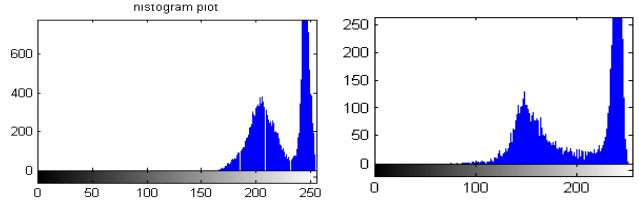


(i) (j)

Figure 5: (i)graph shows the sum of pixel value in opened image as a function of radius of simple RBC (j) graph shows the sum of pixel value in opened image as a function of radius of simple RBC of parasite affected blood cell

In above figure 4, graph for RBC and malaria parasite shows the the sum of pixel values in opned image as a function of radius. Granulometry estimates the intensity surface area distribution of object (parasite affected RBC) as a function of size. Granulometry likens image objects to RBC whose sizes can be determined by sifting them through screens of increasing size and collecting what remains after each pass. Image objects are sifted by opening the image with a structuring element of increasing size and counting the remaining intensity surface area (summation of pixel values in the image) after each opening.We Choose a counter limit so that the intensity surface area goes to zero as we increase the size of our structuring element.

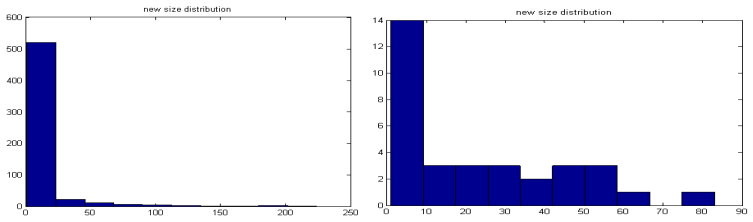
In figure 5, graph for RBC and malaria parasites shows the size distribution or RBC. A significant drop in intensity surface area between two consecutive openings indicates that the image contains objects of comparable size to the smaller opening. This is equivalent to the first derivative of the intensity surface area array, which contains the size distribution of the objects in the image.



(a) (b)

Figure 6: (a) Histogram plot of simple RBC (b) Histogram plot of parasite affected blood cell

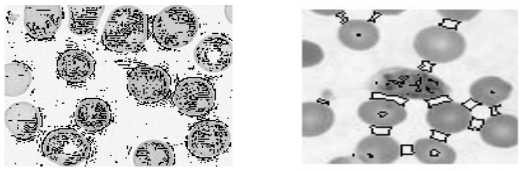
In above figure 6, the threshold gray level for extracting objects of their background. Two threshold level need to determine from the histogram one for erythrocytes and one for parasite. The histogram shows the intensity distribution in image



(a) (b)

Figure 7 :(a)Size distribution of simple RBC cell (b)Size distribution of parasite affected blood cell

In figure 7, histogram containing 10 bins that shows the distribution of different RBC sizes. The histogram shows the most common size for parasite affected RBC in the image. We extract new areas of getting image and update the distribution, here we plots the number of data values that occur in specified data range ,displays data in a Cartesian coordinate system.



(a) (b)

Figure 8: (a)RBC after segmentation (b)Parasite affected blood cell after segmentation

Figure 8 represent the final detected cell of original image. In above figure after comparison the first order statistics we finally indicate the segmented image

VI. CALCULATION CHART FOR SENSITIVITY AND POSITIVE PREDICTIVE VALUE

Observation- The test results of 25 blood images consisting of 502 Red blood cells are included in a table. The values are tabulated below and are compared with manual counting

Table-2

Test images	Algorithm 1		Algorithm 2		Manual counting	
	RBC	Parasites	RBC	Parasites	RBC	Parasites
1	12	2	11	3	12	2
2	12	4	12	3	12	4
3	27	2	27	2	27	2
4	51	7	39	13	51	7
5	0	1	16	2	15	1
6	15	1	11	3	21	1
7	21	1	21	0	17	1
8	0	1	4	3	16	1
9	37	2	12	4	12	2
10	12	2	12	4	12	2
11	24	1	24	0	24	1
12	0	1	11	5	40	2
13	31	2	44	20	12	1

14	0	1	13	0	15	6
15	0	1	48	8	14	1
16	11	6	15	0	17	1
17	0	1	7	1	21	2
18	0	2	17	0	9	2
19	13	1	12	1	25	3
20	25	3	25	7	21	1
21	14	1	14	2	14	0
22	0	2	9	1	14	2
23	21	1	20	0	16	1
24	0	1	10	3	57	2
25	11	1	17	0	8	1

VII. RESULTS FOR SENSITIVITY AND POSITIVE PREDICTIVE VALUE

The performance and accuracy of the algorithm are analyzed using two measures: **sensitivity**, the ability of the algorithm to detect a parasite present; and **positive predictive value** (PPV), the success of the algorithm at excluding non-infected cells. These values are expressed in terms of true positives (TP), false positives (FP) and false negatives (FN):

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

$$PPV = \frac{TP}{TP + FP}$$

According to our result part value of sensitivity comes 98%,and the results of Positive Predictive Value comes 96%, from 25 test images.

Results of first order features -of simple RBC and parasite affected blood cells- ,P.Falciparum, P. Vivax, P.Malerie, P.Oval in this section we can see the first order features are different for each and every parasite

Table-3

ORIGINAL IMAGE	MEAN	SKEWNESS	ENTROPY
Simple RBC	6.8315	-0.6923	1.5342
P.Falciparum	7.2492	-1.0077	1.2036
P.vivax	7.8151	-0.4231	1.3199
P.malerie	7.1696	-0.9730	1.4989
P.oval	7.0041	-0.5615	1.6735

VIII. CONCLUSION

The proposed automated parasite detection algorithm avoids the problems associated with rapid methods, such as being species-specific and having high per-test costs, while retaining many of the traditional advantages of microscopy, viz. species differentiation, determination of parasite density, explicit diagnosis and low per-test costs. On the basis of these results we can differentiate the simple RBC and parasite affected blood cells and also differentiate the species of malaria parasites.

The proposed algorithm is optimized to overcome limitations of image processing algorithms used in the past. Among the tested test algorithms, 'SUSAN edge detection technique' gave good localization of edges but formed a thick border making cell separation difficult. If the staining of RBC is not properly done even than the edge of parasite affected RBC can be easily detected by the help of SUSAN algorithm, this is the important property of SUSAN algorithm. 'Otsu's algorithm' gave accurate separation of RBCs where as local and global thresholding segmented the parasites. Granulometry provides the size distribution of object in image.. The first order features provide the mathematical ranges for simple RBC and parasite affected RBC these values are different for different malarial parasites. Results prove that the algorithm developed in this project has best sensitivity than F.Borey Tek and best positive predictive value than Selena W.S. Sio and F. Borey Tek, and is applicable to many other blood cell abnormalities other than malaria in contrast to the algorithm developed by Jean Phillipe. This is because the percentage of pathological differences in various diseases has very less effect on this robust algorithm. The algorithm detects the species of parasite and

the with a sensitivity of 98% and a positive predictive value of 96%.

IX. FUTURE SCOPE

After successful implementation of the algorithm it can be modified for additional facilities in routine blood check –up like differential white blood cell count, presence of any other parasite causing measure disease, etc.

REFERENCES

[1] World Health Organization. What is malaria? Facts sheet no.94. http://www.who.int/mediacentre/factsheets/fs094/en/.
[2] Foster S, Phillips M, Economics and its contribution to the fight against malaria. Ann Trop MedParasitol 92:391–398, 1998.
[3] F. Castelli, G.Carosi, Diagnosis of malaria, chapter 9, Institute of Infectious and Tropical Diseases, University of Brescia (Italy).
[4] Jean-Philippe Thiran, Benoit Macq, Morphological Feature Extraction for the Classification of Digital Images of Cancerous Tissues. IEEE Transaction on Biomedical Engineering, Vol. 43, no. 10, October 1996.
[5] C. Di Ruberto, A. Dempster, S. Khan, and B. Jarra. Automatic thresholding of infected blood images using granulometry and regional extrema. In ICPR, pages 3445–3448, 2000.
[6] Silvia Halim, Timo R. Bretschneider, Yikun Li, Estimating Malaria Parasitaemia from Blood Smear Images. 1-4244-03421/06/$20.00 ©IEEE, ICARCV 2006.
[7] Selena W.S. Sio, Malaria Count: An image analysis-based program for the accurate determination of parasitaemia, Laboratory of Molecular and Cellular Parasitology, Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore. May 2006.
[8] F. Boray Tek, Andrew G. Dempster and Izzet Kale, Malaria Parasite Detection in Peripheral Blood Images, Applied DSP & VLSI Research Group, London, UK, Dec 2006.
[9] Rafeal C. Gonzalez, Richard E. Woods, Digital Image Processing, 2nd Edition, Prentice Hall, 2006.
[10] S. M. Smith, J. M. Bardy, SUSAN—A New Approach to Low Level Image Processing, International Journal of Computer Vision, Volume 23, and Issue 1 Pages: 45 – 78, may 1997.
[11] Di Ruberto C, Dempster A, Khan S, Jarra B, Analysis of infected blood cell images using morphological operators. Image Vis Comput 20(2):133–146, 2002.
[12] Mui JK, Fu K-S, Automated classification of nucleated blood cells using a binary tree classifier. IEEE Trans Pattern Anal Machine Intell 2(5):429–443, 1980

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