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Machine Counting of Malaria Infected Blood Cells Using RGB Images

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ABSTRACT

The most widely used laboratory confirmation technique for malaria is visual inspection of Giemsa stained blood smears on microscope. A detection and counting method for malaria infected blood cells in a colour (RGB) microscopic image was developed with the help of machine vision and artificial neural networks (ANN). The developed system is capable of detecting individual blood cells in the image and recognized them as malaria infected or non-infected. The system is capable of producing the number of blood cells in each category, which can be used as an indicator of severity of infection. The system was trained for 40 blood cells (from seven images) manually marking them as infected or non-infected, and 120 blood cells (from 15 images) were used to test the system. The sensitivity and the specificity of the system for that data set was found to be 90.0 % and 95.7 % respectively for the images of blood cells of malaria infected and uninfected by *Plasmodium falciparum parasites.*

1. INTRODUCTION

Malaria is a severe infectious disease caused by *Plasmodium* parasites, which spread through infected females of *Anopheles* mosquito vectors. Malaria can be fatal unless diagnosed and treated promptly. According to 2013 Wold Health Organisation (WHO) report on malaria [1], an estimate of 627,000 malaria deaths have occurred out of 207 million malaria cases which were reported in 2012 and about 3.4 billion people are at risk of malaria. *Plasmodium falciparum* is the parasite, which causes most severe form of malaria out of five identified *Plasmodium* parasites [2]. Most importantly, deaths due to malaria can be prevented by early detection and treatment. It has been noted that the more steps are taken to prevent and control malaria, the less malaria cases are reported [1].

Clinical diagnosis and microscopic diagnosis are the two main approaches of diagnosis of malaria. Clinical diagnosis, the most used method is not reliable since the symptoms of malaria are unspecific [3]. The most widely used field technique for laboratory confirmation of malaria is microscopic inspection of Giemsa stained blood smears [3]. Although, this technique is very cheap, if there is a skilled person for analysis, it is capable of detecting *Plasmodium* species. The main disadvantages of this technique are the requirement of a well-trained skilled person for analysing the sample through microscope and requirement of a long time for analysis. Hence, the results on the skill level of the person who analyse the sample and it does not allow running multiple tests simultaneously [4]. Rapid diagnostic tests (RDTs) are the next practically possible laboratory test for confirming malaria infection. RDTs can be carried out by a person with

minimum skills with the help of commercially available kits without requirement of electricity or any other specific equipment. The main disadvantages of RDTs are lack of sensitivity at low-level presence of parasites in blood, inability of quantifying parasite density and inability of differentiating different *Plasmodium* parasite species [3, 4]. Moreover, the cost associated with a RDT test is relatively high when comparing with the microscopic technique.

Although, unwanted use of antimalaria can cause side effects and is a heavy cost for communities, prescription of such drugs are common practise in countries where malaria is endemic [5,6,7]. Proper use of laboratory diagnostic tests such as microscopy and RDT can reduce irrational use of antimalaria drugs, which indirectly facilitates reach of such drugs to most required patients [7].

Machines can be trained with proper algorithms to perform routine work like visual inspection and detection of malaria-infected blood cells. Once the system is trained properly, it can perform routine work very quickly. Artificial neural networks (AANs) are good computational models, which perform well in supervised learning algorithms where expert knowledge should be provided with separately [8, 9, 10].

This article presents a method to make use of conventional microscopy on stained thin blood smears for a fast, accurate and inexpensive mechanism identification of *Plasmodium falciparum* at life stages of ring-form troposites, mature troposites and schizonts infected blood cells malaria diagnosis with the help of machine vision and machine learning.

2. MATERIALS AND METHODS

2.1 Image Collection

The scope of this study is limited to inspecting Microscopic RGB images of stained thin blood smears of *Plasmodium falciparum* infected blood cells at ring-form troposites, mature troposites and schizonts of parasites' life stages. Relevant images were downloaded from Centre for Disease Control (CDC) website (http://www.cdc.gov/dpdx/malaria/gallery.html). The spatial resolution of those images is 300 × 300 pixels.

2.2 Image Pre-processing and Segmentations

Although the selected images were having same number of pixels, the intensity levels are different one from the other as those images have been acquired using different setups with different brightness, gain and exposure time. As such, the main intention of pre-processing is to remove salt and pepper noise in the images. A 5 × 5 median filter was used to remove the salt and pepper noise.

The segmentation stage used a combination of image processing methods for border and region detection in order to reject the image background by identifying each of the objects present in the processed image. Histogram thresholding was used to segment thin blood smear images into its constituent regions. The goal of thresholding is to locate blood cells and boundaries (curves and lines) in an image. Green colour layer of input RGB images was used for segmentation, since; green colour component is the least noisy component of RGB image of malaria infected blood thin smears [11]. Otsu algorithm [12] was used to find the threshold value of intensity histograms of the green layer of the RGB images (see Fig. 1: A).

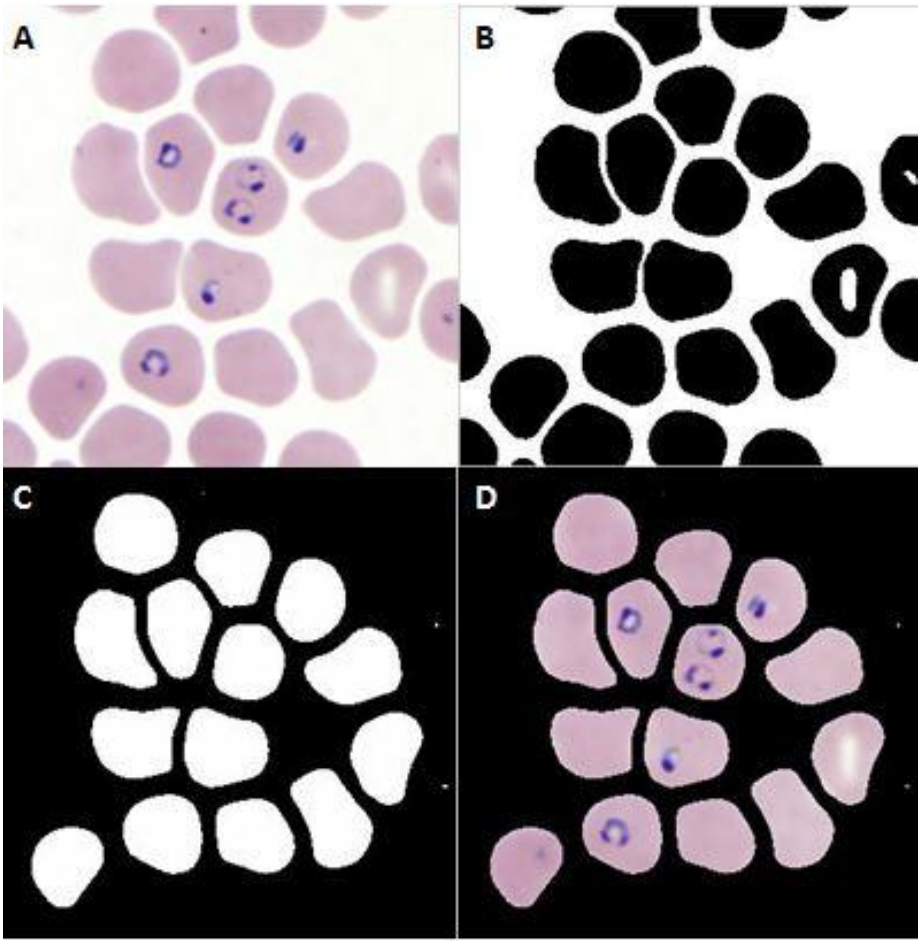

Fig. 1: A) original image, B) resultant binary image of histogram thresholding obtained from green colour component of input RGB image, C). Test result of performing erosion operation on close edges filled image, D). Resultant image of performing AND operation, connected components labelled image with Red, Green and Blue colour components of input image, and combining the resultant R, G and B components.

Usually, central portion of red blood cells appear lighter compared to the border, when images are taken in transmission mode, due to the lower optical path length in centre portion (red blood cells are biconcave) [13]. Hence, when segmented using intensity

histogram thresholding techniques, some unfilled islands can be seen (see Fig. 1: B). Erosion operation is performed using diamond shaped morphological structuring element in order to fill those unfilled islands (see Fig. 1: B). By performing AND operation (algebraic multiplication) of individual pixels of the filled segmented binary image with each layer of the RGB image, red blood cells can be identified (see Fig. 1: D). The segmented images of blood cells were isolated considering the width, height and area of the detected object and their size is normalised to 30 × 30 pixels.

2.3 Feature Extraction for Machine Learning

The main feature considered in this study is the colour of each pixel belong to a selected red blood cell. Due to the irregularities in the sample, light intensities of the captured image are changed from place to place, and the experimental data have to be corrected in order to minimize the influence of this effect on the findings. Red, green and blue colour vectors were considered as axes of a Cartesian space where red, green and blue vectors were mapped to x, y and z axes. Then the coordinates were transformed to spherical coordinates (r, θ, φ) (see Fig. 2).

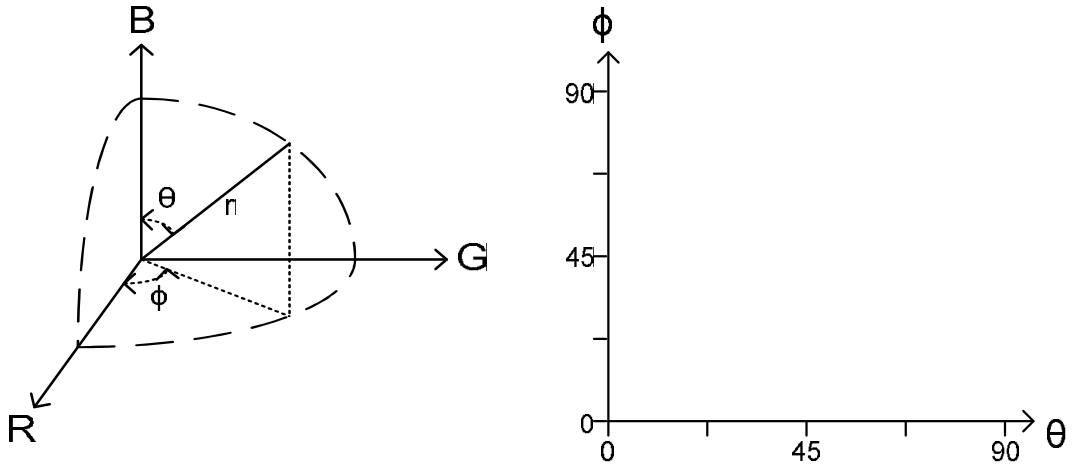

Fig. 2: Conversion of the red (R), green (G) and blue (B) from the Cartesian coordinates to spherical coordinates r, θ and ϕ; Right: New spectral space generated by considering θ and ϕ disregarding r which carried intensity information.

In spherical coordinate system, r represents the intensity of a pixel and θ and φ can be considered as ratios of the colours. In order to eliminate the geometrical dependency, r was neglected. The θ and φ values of each pixel give spectral information and the range of those θ and φ are in $[0, \frac{\pi}{2}]$ range. Then a two dimensional (2D) histogram of θ and φ with 11 × 11 bins was constructed, and subsequently, the 2D histogram was converted into a single row vector by placing each row after another row for convenience of analysis. The single row vectors created for each sample were used as a feature set of each blood cell image for training the artificial neural network. Expert matrix for each feature set was constructed by manual visual inspection of images for the training set.

2.4 Design and Training of ANN

Feed forward back-propagation artificial neural network used to classify blood cell images in to malaria infected or non-infected. Feature vector of every blood cell image has column matrix with 121 elements together with the corresponding element of expert matrix there are 121 inputs for ANN. The number of required hidden layer neurons were calculated using the geometrical mean of the number of neurons in input and output layers, and it was found that required number of hidden layer neurons in ideal case was is 15.

The designed ANN was trained by changing its minimum error, learning rate, momentum, number of neurons on each of the layers. It was found that the best number of hidden layer neurons required is 10, learning rate is 0.6, momentum is 0.3 and minimum error is 1×10^{-10} when ANN was giving good classification. Fig. 3 shows the artificial neural network diagram of the designed ANN.

Six images of thin blood smears has used as the training set. The system recognized 40 different blood cells and infected and non-infected blood cells marked manually for creating expert matrix.

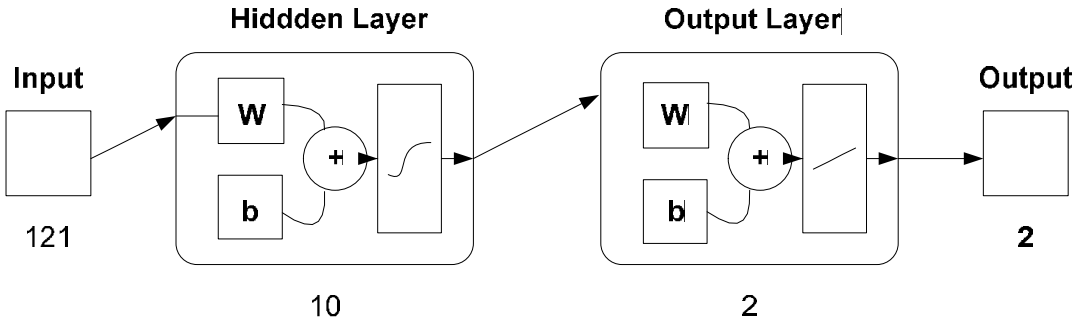

Fig. 3: Neural network diagram of 121 inputs, two outputs with 10 hidden layers, which was used to train the artificial neural network for classification parasites infected blood cells and not infected blood cell images.

2.5 Testing the Samples

Fifteen images of thin blood smears were fed to the system for testing the performance. Upon completion of the processes, the system marked identified red blood cells as infected or non-infected in red and green circles as shown in Fig. 4.

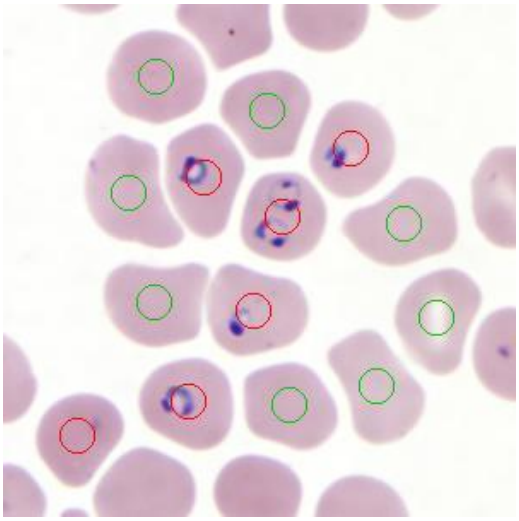

Fig. 4 System marked infected (in red circles) and non-infected (in green circles) blood cells on one of the images fed to the system for analysing. Note that, cells appearing partially in the edges have not been considered by the system, and in this particular case, there are no false positives or false negatives.

3. RESULTS AND ANALYSIS

The system produced images manually inspected for determining the quality of classification. By manual inspection of images produced by the system (such as Fig. 4), it was found that 50 cells were marked as infected and 70 cells were marked as non-infected out of 120 recognized cells. The performance of the system in terms of number of true negatives, true positives, false negatives and false positives are tabulated in Table 1. The calculated sensitivity and the specificity according to Table 1 are 90.0 % and 95.7 % respectively.

Table 1: System performance on test data: it has been identified that out of 120 system-detected cells, 50 cells were infected and 70 cells were non-infected.

	True positives (Out of 50)	True negatives (Out of 70)	False positives (Out of 70)	False negatives (Out of 50)
Number of cells	45	67	3	5
Percentage	90 %	95.7 %	4.3 %	10 %

4. DISCUSSION AND CONCLUSIONS

The proposed automated method includes an effective and an efficient method for counting malaria infected and not infected blood cells. The system works quick and very effective compared to the traditional approach. The performance of this artificial neural network can find in Fig. 5. As shown in Fig. 5, validation and test curves are very similar and validation curve increased significantly before the testing curve increased. Therefore, it is possible to conclude that the training has been performed properly.

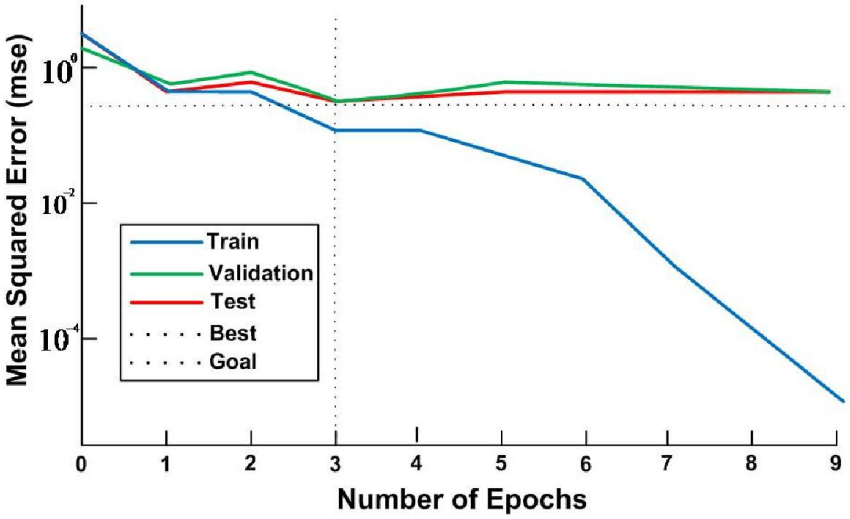

Fig. 5: The performance (or behaviour of the mean square error) of the network when number of epochs are increased. Note that the validation and the test curves are very similar and validation curve increased significantly before the testing curve increased.

The overall performance of the network is 94 %. In training, testing and validation phases, accuracy of classify to first class (parasites infected blood cells) is 91 % and accuracy of classify to other class (parasites non infected blood cells) is 95 %.

The developed method can be used to process real time data acquired though the imagers attached to the microscopes. The main feature used in this technique is the colour ratio of a typical tri-band (RGB) imager. As such, even a basic type of microscope can be converted in to a compatible device by attaching a typical colour (RGB) imaging sensor. In addition, by changing emission wavelength of the light source with the used of Light Emitting Diodes (LEDs), system sensitivity can be improved [14]. Although the calculated sensitivity and the specificity of the system are 90.0 % and 95.7 % respectively, it can be improved by categorising the detected blood cells in to groups in relation to the abnormal shapes and different sizes.

Since the system requires a digital microscope or a typical microscope with camera attachment, an additional computer is needed to operate the system. This could make the system more expensive and difficult to use in areas where there is no electricity. Use of a single board computer would be a remedy for this drawback.

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REFERENCES

1. World Health Organization, *World malaria report,* WHO, (2013)
2. Centres for Disease Control and Prevention, *Global Health - Division of Parasitic Diseases and Malaria,* Online: CDC, (2012), http://www.cdc.gov/
3. World Health Organization, *New Perspectives-Malaria Diagnosis,* WHO/USAID informal consultation, (1999)
4. Lema, O. E., Carter, J. Y., Nagelkerke, N., Wangai, M. W., Kitenge, P., Gikunda, S. M., Arube, P. A., Munafu, C. G., Materu, S. F., Adhiambo, C. A. And H. K. Mukunza, *Comparison of five methods of malaria detection in the Outpatient setting,* Am. J. Trop. Med. Hyg., 60, (1999) 177-82
5. Khan, S. Y., Khan, A., Arshad, M., Tahir, H. M., Mukhtar, M. K., Ahmad., K. R. and Arshad, N., *Irrational use of antimalarial drugs in rural areas of eastern Pakistan: a random field study,* BMC Public Health, 12, (2012) 941:1-6
6. Ezenduka, C. C., Ogbonna, B. O., Ekwunife, O. I., Okonta, M. J. and Esimone, C. O., *Drugs use pattern for uncomplicated malaria in medicine retail outlets in Enugu urban, southeast Nigeria: implications for malaria treatment policy,* Malaria Journal, 13, (2014) 243:1-10
7. Malik, M., Hassali, M. A. A., Shafie, A. A. and Hussain, A., *Why Hospital Pharmacists Have Failed to Manage Antimalarial Drugs Stock-Outs in Pakistan: A Qualitative Insight,* Malaria Research and Treatment, 2013, (2013) 1-9
8. Kursk, S. M., Rasras, R. J., and Skopin, D., *The artificial neural network based approach for mortality structure analysis.* American Journal of Applied Sciences, *3*(2), (2006) 1698
9. Kasmin, F., Prabuwono, A. S., & Abdullah, A., *Detection of leukemia in human blood sample based on microscopic images: a study*. Journal of Theoretical & Applied Information Technology, *46*(2), (2012) 579-585
10. Hänscheid, T., Melo-Cristino, J., & Pinto, B. G. Automated detection of malaria pigment in white blood cells for the diagnosis of malaria in Portugal. *The American journal of tropical medicine and hygiene*, *64*(5) (2001) 290-292.
11. Ross, E. N., Pritchard, J. C., Rubin, M. D. and Dusé G. A., *Automated image processing method for the diagnosis and classification of malaria on thin blood smears,* Med. and Bio. Eng. and Comp., 44, (2006) 427-436.
12. Otsu, N., *A threshold selection method from gray-level histograms*, Automatica, 11, (1975), 23-27
13. Potter, A. and Smith, M., *A., Summary Study of Blood,* Online: Microscopy UK, (1995), http://www.microscopyuk.org.uk/
14. Brydegaard, M., Merdasa, A., Jayaweera, H., Ålebring, J. and Svanberg, S., *Versatile multispectral microscope based on light emitting diodes*, Review of Scientific Instr., 82, (2011) 123106