An Intelligent Recognition Procedure for Trophozoite Stages of Plasmodium Knowlesi Malaria

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***Abstract*—Plasmodium (P.) Knowlesi is a fifth species of the malaria parasite in the world that caused a serious health problem. Current information suggests that P. Knowlesi is spread to people when an Anopheles mosquito infected by a monkey then bites and infects human (zoonotic transmission). Early identification of P. Knowlesi Malaria is an important step to an effective treatment. P. Knowlesi Malaria identification process is usually carried out with a 100x magnification of thin blood smear using microscope observation. However, this process is time-consuming and very tedious and strenuous for the human eyes. It also has a problem to differentiate between trophozoite, positive control (schizont and gametocyte) and negative control (white blood cell (WBC) and artefact). To overcome these situations, a computer-aided diagnosis system is developed to automatically identifying trophozoite stages of P. Knowlesi Malaria as early identification species, positive control and negative control. The processes involved starting from image acquisition, image processing and recognition. For image processing method, the most important part is the segmentation where the Otsu's method is utilised to obtain the region of interest (ROI) of the infected cell. The features consist of the size of infected cell and size of the object. Finally, the recognition method using Multilayer Perceptron (MLP) is applied. The results show that the proposed automatic procedure is capable of recognising the trophozoite stage of P. Knowlesi Malaria with an accuracy of 98.70% for training and 97.67% for testing, using MLP trained by Lavernberg Marquardt (LM).**

***Index Terms*—Image Processing; MLP; P. Knowlesi Malaria; Recognition; Thin Blood Smears**

I. INTRODUCTION

Malaria is the cause of perilous health problem in the world with around 212 million cases and 429000 deaths of malaria cases in 2015 [1]. Malaria is a mosquito-borne infectious disease caused by parasitic protozoans. This parasite is a type of Plasmodium that infected human and animals only. Infected female Anopheles mosquito will transmit the parasite from the infected host into the human bloodstream. Malaria parasite can be classified into five species which are P. Falciparum, P. Vivax, P. Malariae, P. Ovale and P. Knowlesi [2]. It has been reported that funding for controlling and eliminating malaria globally in 2015 was totalled to US$ 2.9 billion, and US$ 6.4 billion annually to achieve the global target by 2020 [1].

In Malaysia, the highest occurrence of malaria has been found in rural and forest areas [3]. The Ministry of Health (MOH) has taken various measures to eliminate malaria cases in the country by 2020 completely. However, efforts to eliminate malaria for four malaria species in Malaysia namely P. Falciparum, P. Vivax, P. Malariae and P. Knowlesi never blocking P. Knowlesi to spread, where 72% has been reported P. Knowlesi cases in Malaysia of the 2985 local cases [3]. The life cycle of P. Knowlesi that takes only 24 hours to be a vector (gametocyte stage) has accelerated the dissemination process of P. Knowlesi. The transmission process of P. Knowlesi is different with other malaria parasite species where P. Knowlesi is caused by the female Anopheles mosquito as the transmitter that passes the parasite taken from long-tailed macaques to human (zoonotic transmission) [4]. Early stage identification of P. Knowlesi is an important factor to be a part of prevention and control the spread of P. Knowlesi.

Currently, there are two methods to identify malaria parasite namely; microscopic and non-microscopic diagnosis [4]. The thin blood smears test is used to determine the stages or species of malaria parasite. Clinically, malaria is diagnosed visually by medical experts using microscopic images. However, the visual inspection by human perception is subjective and occasionally exposed to error due to the weakness of images that have a lot of noise, unwanted artefacts, and blurring effect (due to an old microscope) [5]. Therefore, efforts have been intensively carried out to improve conventional diagnosis methods (i.e. the manual method by a pathologist) using computer-aided systems.

Based on the above facts, many computer-aided systems have successfully been developed to assist the diagnostic procedure and reduce the percentage of error due to visual inspection by a human. The identification process also will be effective and easy to be used. Several techniques have already been developed and applied for the automatic recognition for malaria parasite by several researchers in their studies [6-13] using image processing algorithm.

I. Suwalka et al. [6] only compare either positive malaria or negative malaria in the image based on the extracted components of the parasite without identifying by stage of malaria and negative malaria (WBC, artefact). N. A. Khan et al. [7] proposed an automated diagnosis by considering four species of malaria namely P. Falciparum, P. Vivax, P. Malariae and P. Ovale. However, they used manual thresholding to all images of malaria parasite which is not considering the various staining colour problems on the image. A. Mehrjou and T. Abbasian [8] used the colour translation for b* from L*a*b* channel after select colour

plane g (green) channel and proceeded using a clustering method to cluster the image. Unfortunately, their segmentation method only focuses on the colour of parasite and may be over-segmented with negative malaria (WBC & artefacts) due to similar colour among WBC, artefacts and parasite. H. A. Nugroho et al. [10] proposed classification process for Plasmodium Falciparum species. However, they only process the ROI of 150 x 150 cropping image only without consider the original size of the image that captured from microscopic image to analyse and identify the image. This situation may affect the identification of negative malaria that cannot be identified properly.

Thus, this study proposes an intelligent recognition procedure for trophozoite stages of P. Knowlesi by considering positive control (i.e. schizont and gametocyte) and negative control (i.e. WBC and artefacts) that have in P. Knowlesi thin blood smear image using a microscope. The main objective is to preserve the size and shape of the malaria parasite in the infected cell. The system is developed to recognise the trophozoite stages of P. Knowlesi Malaria, positive control and negative control using image processing techniques including filtering, contrast enhancement, RGB to Grayscale, segmentation, feature extraction and recognition process using LM-MLP to classify the image.

This paper is arranged as follows: in section 2, the methodology to develop an automated recognition is explained. Section 3 describes the application of the method and experimental results are presented and analysed. Finally, section 4 provides the conclusion of this work.

II. METHODOLOGY

Malaria samples were prepared by experts from Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan. One of five species known as P. Knowlesi was used as a source for parasite samples of this study due to its sudden spread of this new species in Malaysia over the years. P. Knowlesi in trophozoite stages was considered to recognise as early spread stage. An automated recognition procedure for trophozoite stages of P. Knowlesi Malaria is proposed according to the following flowchart in Figure 1.

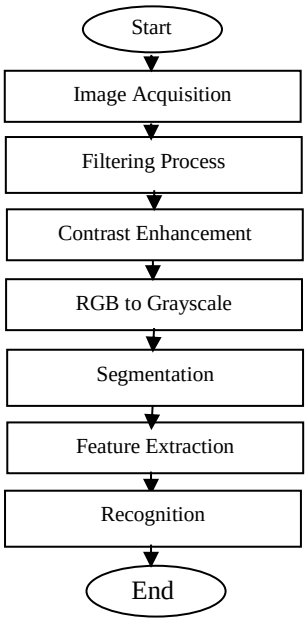


Figure 1: Flowchart of an Intelligent Recognition

Procedures for trophozoite stages of P. Knowlesi Malaria are as follows.

A. Image Acquisition

Images are acquired by using high-resolution digital Olympus camera XC50 to microscope BX41. Thin Blood Smear Images are captured under 100X magnification resolution using the aid of immersion oil. This study only focuses on differentiating between trophozoite stages of P. Knowlesi, positive control (i.e. schizont and gametocyte) and negative control (i.e. WBC, and artefact). 300 thin blood smear images including 100 images of P. Knowlesi in trophozoite stage, 50 images of schizont, 50 images of gametocyte, 50 image for WBC and 50 images of the artefact. Images were saved in the BMP format in 574 x 764 in colour (red, green blue (RGB)) images.

B. Filtering Process

In image processing, the filtering process is to eliminate the distortion of noise, eliminate unwanted information and improve the quality of the image [15]. For the information of the malaria parasite images, artefact and other noise in the x100 magnifier image is considered small and more alike as salt and pepper noise. Hence, the colour median filter is selected for this filtering process because this is the judicious method to remove salt and pepper noises at the same time retain the original image data [16]. Colour median filter compare neighbour pixels, pixels with a slightly different value within the tolerance range will be kept while the huge pixel value different will be discarded. In this process, neighbour pixels, n x n (n=7) has been used to yield a better smoothing effect in colour images.

C. Contrast Enhancement

The P. Knowlesi in the infected RBC images (ROI) that have been captured by the microscope is blur and poor in quality. Hence, colour contrast enhancement needs to apply on the image to enhance the quality of the ROI image. Modified global contrast stretching (MGCS) has been selected in this study to refine the contrast of the overall images, especially ROI images [17]. MGCS with linear mapping function manages to preserve the colour structure and keep the information of the original image at the same time bring up the desired colour of the image. In this process, the pixels between the minimum threshold value and maximum threshold value will undergo a stretching process to map into new range within new minimum stretching value and new maximum stretching value. The pixel out of the range of this stretching process will all undergo a compression process. Figure 2 explains the MGCS stretching process.

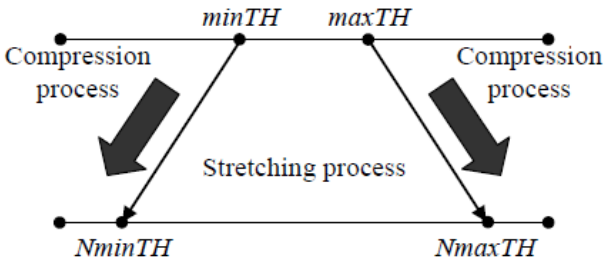


Figure 2: Modified Global Contrast Stretching

D. RGB to Grayscale

In this paper, the RGB image was converted into a grayscale image by forming a weighted sum of the R, G, and B components [21]:

$$I = 0.2989 * R + 0.5870 * G + 0.1140 * B \quad (1)$$

The purpose of the RGB image converted to grayscale is to simplify the image divided into two regions (i.e. black and white). This is to facilitate segmentation methods as in step E.

E. Segmentation

Segmentation consists subdivided and partitioning of an image into the desired regions. Image segmentation extracts the ROI based on the similarity and dissimilarity [18]. To obtain the good segmentation in this study, steps have been successfully done to obtain the ROI by considering as follows:

1. Load the grayscale image from part D.
2. Segment the grayscale image from step 1 into a binary image using Otsu's method [9]. Foreground represented as white (assigned to 1) and background represented as black (assigned to 0) to display the cell images.
3. Filling the hole in the foreground as 1 in the segmented image in step 2.
4. Remove the unwanted small object in step 3 (we set as less than 100 pixels) using morphological operation.
5. Retrieve the grayscale image from step 1 for foreground region and change background region to white by assigned the background region to 255.
6. Segment image in step 5 using manual thresholding method by setting 0 to 200 intensity values to be 1 and others is 0 to display the object image. The equation as follows:

$$I(x,y) = \begin{cases} 1, & \text{if } 0 < I(x,y) < 200 \\ 0, & \text{if } I(x,y) \geq 200 \end{cases} \quad (2)$$

7. Identify which object in step 6 overlapped with the cell in step 4, the rest will be deleted.
8. Infected cell together with the object will be displayed.

Automated segmentation methods have been successfully developed. In this segmentation process, images are divided into two considerations, namely segmentation based on cell and segmentation based on object images. Step 2 to 4 are the segmentation process of the cell images. Step 5 to 6 are the process for obtaining object images. Lastly, the consideration of the two steps (i.e. step 7 and 8) will overlap together to get the infected cell only.

During the segmentation process, the unwanted small objects are exposed in the image. Therefore, the unwanted small object has been removed in the image. If a small object less than 100 pixels of the image in step 4 then the small object will be removed from the image. This is because the small objects are characterised as noise. It has also been agreed upon by the microbiologists during the experiment. For step 6, manual thresholding method that has been set 0 to 200 intensity value has been used to segment the object in the image. This intensity value has been selected based on observation from microbiologist for 300 images during the experiment.

Figure 3 shows the process of an image in image processing method starting from the original image, median filtering, contrast enhancement, RGB to grayscale, and segmentation process.

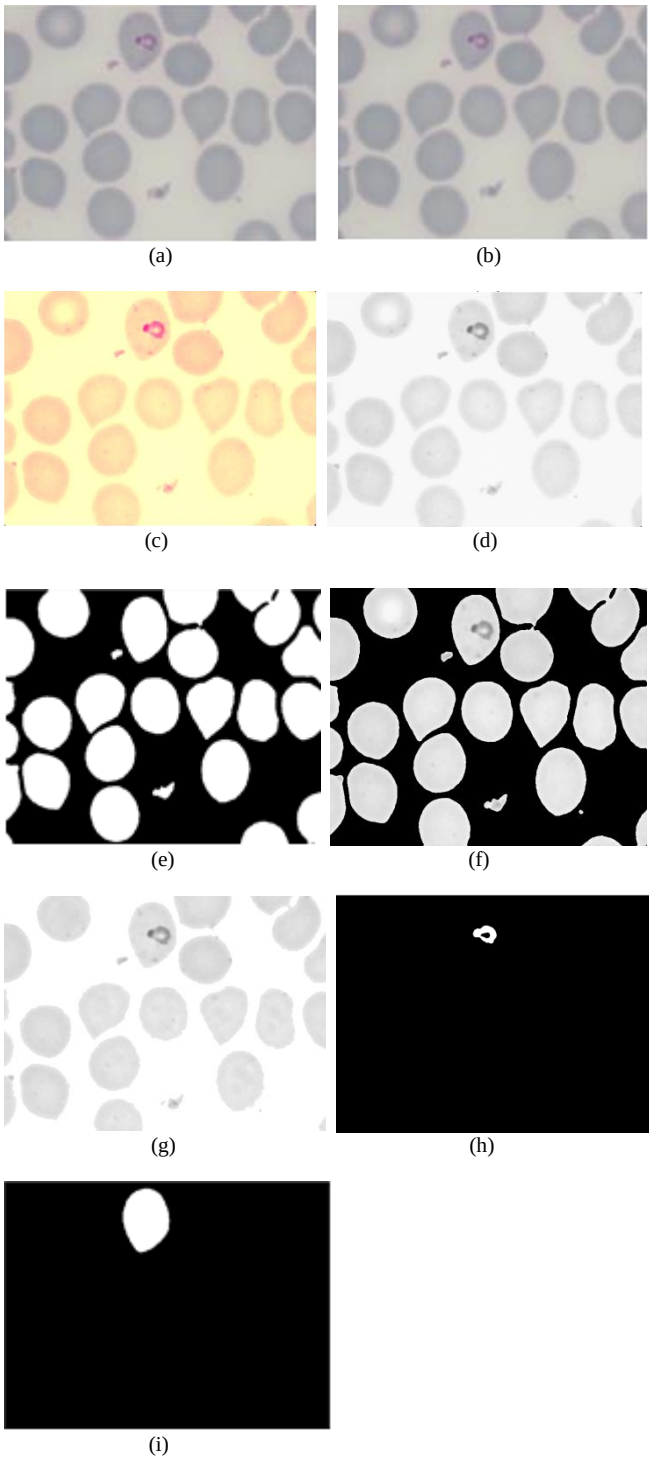
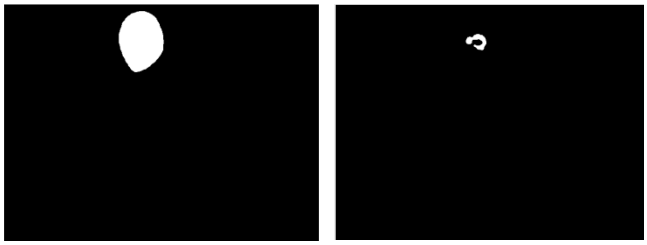


Figure 3: P. Knowlesi in Trophozoite stage Image ; a) Original Image, b) Result of Median Filtering Image, c) Result MGCS Contrast Enhancement Image, d) Result of RGB to Grayscale Image, e) Result of Otsu's Method (Segmentation) Image, f) Result of grayscale image after segmentation, g) Result of step 5, h) Result of object, i) Result of infected cell.

F. Feature Extraction

The feature extraction is an important part of classifier because it enables us to identify and recognise the image based on their relevant feature. Size of infected cell and size

of the object has been used as features in this project to obtain the good accuracy during the recognition process. The process to measure the size of infected cell and size of the object are based on the sum of the white pixel as shown in Figure 4, (a) and (b) respectively.



(a) (b)

Figure 4: Feature Extraction for P.Knowlesi in trophozoite stage; a) Size of infected cell image, b) Size of object

The range of size of infected cell and size of the object is shown in Table 1. We can see that size of infected cell and size of the object have an overlapping range of each class. However, both of these features will cover this situation to differentiate each other and obtain a good result.

Table 1
The Range of Feature Extraction Parameter

Object	Range	
	Size of infected cell	Size of object
P. Knowlesi in trophozoite stages	6082 ~ 18323	1097 ~ 4400
Positive Control	7324 ~ 14951	4576 ~ 13941
Negative Control	1731 ~ 69619	1699 ~ 67023

G. Recognition

Recognition or classification is a process where the image will be classified into different categories. In this process, it is very important to ensure the detection of Plasmodium Knowlesi parasite in the image. MLP was selected to be used in the Artificial Neural Network (ANN) classification. Training classifier is used to utilise the system so that the trophozoite stage of P. Knowlesi, positive control and negative control can be identified clearly. Training input will be supplied into the input while the coefficient will be adjusted to suit the output based on the target within the input feature. Levenberg-Marquardt (LM), Bayesian Regulation (BR) and Resilient Backpropagation (RP) were the algorithms that had been used to train and test for the accuracy and robustness of the result [20].

III. Experimental Result and Discussion

All data from feature extraction process has been analyzed for the recognition process using Multi-Layer Perceptron (MLP). 300 images were used as sample data which consist of 100 images of P. Knowlesi in trophozoite stages, 50 images of schizont stages, 50 images of gametocyte stages, 50 images of WBC and 50 images of the artefact. Two features extracted from the size of infected cell and size of the object was used as the input for each learning algorithm of Bayesian Regulation (BR), Lavernberg Marquardt (LM), and Resilient Backpropagation (RP) [20].

The data of the images were designed to be a different set of training and testing data to enhance the robustness of the result. Table 2, 3 and 4 show the result using a different type of training and testing set which is 90% training 10% testing, 80% training 20% testing and 70% training 30% testing.

Based on overall data of multilayer perceptron in Table 5, the conclusion can be made where the highest average accuracy among the other learning algorithms of the classifiers is Multilayer Perceptron (MLP) of Levenberg-Marquardt (LM) learning algorithm. The results show 90% training 10% testing which has 97.67% correct testing accuracy (acc.) performance with the least value of error 2.33% compared to the others learning algorithm.

Table 2
Data Set for 90% Training 10% Testing of Three types of Learning Algorithm

MLP	Training Acc.	Testing Acc.
LM	98.70	97.67
BR	98.26	97
RP	94.47	91.2

Table 3
Data Set for 80% Training 20% Testing of Three types of Learning Algorithm

MLP	Training Acc.	Testing Acc.
LM	96.94	95.90
BR	96.90	90.20
RP	93.45	90

Table 4
Data Set for 30% Training 70% Testing of Three types of Learning Algorithm

MLP	Training Acc.	Testing Acc.
LM	96.34	94.18
BR	95.02	92.49
RP	96.02	92.67

Table 5
Overall Data of the Multilayer Perceptron (MLP)

MLP	90% Training Acc.	10% Testing Acc.	80% Training Acc.	20% Testing Acc.	30% Training Acc.	70% Testing Acc.
LM	98.70	97.67	96.94	95.90	96.34	94.18
BR	98.26	97	96.90	90.20	95.02	92.49
RP	94.47	91.20	93.45	90	96.02	92.67

IV. Conclusion

An Intelligent Recognition Procedure for Trophozoites Stages of P. Knowlesi Malaria has been successfully analysed using image processing method and recognised using MLP to get the performance of the data. The objective is to develop an automated recognition of P. Knowlesi Malaria in Trophozoite stages, positive control and negative control stages of thin blood smear images in positive P. Knowlesi samples slide were achieved using image processing method. From image processing method, the process of contrast enhancement, segmentation and feature extraction is used to obtain the characteristic of the image. The experimental results have been done using MLP (LM) to get the good accuracy of recognition. The procedure proposed helps in recognition for trophozoite stages of P. Knowlesi Malaria

with an accuracy of 98.70% for training and 97.67% for testing.

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REFERENCES

[1] World Health Organization, Malaria Report 2016.

[2] D. Anggraini, A.S. Nugroho, C. Pratama, I.E. Rozi, A.A. Iskandar, R.N. Hartono, "Automated Status Identification of Microscopic Images Obtained from Malaria Thin Blood Smears", *in Proc of 2011 International Conference on Engineering and Informatics, ICEEI IEEE,* pp 1-6, 17 – 19th July 2011.

[3] Country Briefing,UCSF Global Health Group's Malaria Elimination, Eliminating Malaria in Malaysia, March 2015.

[4] S.B. Millar, J. Cox-Singh, "Article-Review of Human Infection with Plasmodium Knowlesi – zoonotic malaria", *Clinical Microbiology & Infection*, vol. 21 (7), pp. 640-648, July 2015.

[5] S.N.A.M. Kanafiah, Y. Jusman, N.A.M. Isa, Z. Mohamed, "Radial-Based Cell Formation Algorithm for Separation of Overlapping Cells in Medical Microscopic Images", *in Procedia Computer Science of International Conference on Computer Science and Computational Intelligence (ICCSCI 2015)*, vol. 59, pp. 123-132, 2015.

[6] I. Suwalka, A. Sanadhya, A. Mathur, M.S. Chouhan, "Identify Malaria Parasite Using Pattern Recognition Technique", *in Proc. Of 2012 International Conference on Computing, Communication and Applications,IEEE* pp. 1-4, 2012.

[7] N.A. Khan, H. Pervaz, A.K. Latif, A. Musharraf, Saniya, "Unsupervised Identification of Malaria Parasites using Computer Vision", *in Proc. 2014 11th International Joint Conference on Computer Science and Software Engineering (JCSSE),IEEE,* pp. 263-267, 2014.

[8] A. Mehrjou, T. Abbasian, "Automatic Malaria Diagnosis System", *in Proc. of 2013 RSI/ISM International Conference on Robotic and Mechatronics (ICROM),IEEE*, pp.205-211, 2013.

[9] S.S. Savkare, S.P. Narote, "Automated System for Malaria Parasite Identification", *in Proc. of 2015 International Conference on Communication, Information & Computing Technology (ICCICT), IEEE*, pp.1-4, Jan. 16-17th, 2015.

[10] H. A. Nugroho, S.A. Akbar, E.E.H. Murhandarwati, "Feature Extraction and Classification for Detection Malaria Parasites in Thin Blood Smear", *in Proc. of 2015 International Conference on Informatic Technology, Computer and Electrical Engineering (ICITACEE), IEEE*, pp. 197-201, Oct. 16-18th, 2015.

[11] H.A. Mohammed, I.A.M. Abdelrahman, "Detection and Classification of Malaria in Thin Blood Slide Images", *in Proc. of 2017 International Conference on Communication, Control, Computing and Electronics Engineering (ICCCCEE), IEEE*, pp. 1-5, 2017.

[12] P. Rakshit, K. Bhowmik, "Detection of presence of Parasites in Human RBC in Case of Diagnosis Malaria using Image Processing", *in Proc. of 2013 IEEE Second International Conference on Image Information Processing (ICIIP)*, pp. 329-334, 2013.

[13] W. Preedanan, M. Phothissonothai, W. Senavongse, S. Tantisatirapong, "Automated Detection of Plasmodium Falciparum from Giemsa-Stained Thin Blood Films", *in Proc. of 2015 8th International Conference on Knowledge and Smart Technology (KST), IEEE*, pp. 215-218, 2015.

[14] D. Anggraini, A.S. Nugroho, C. Pratama, I.E. Rozi, V. Pragesjvara, M. Gunawan, "Automated Status Identification of Microscopic Images Obtained from Malaria Thin Blood Smears using Bayes Decision: A study case in Plasmodium Falciparum", *in Proc. Of 2011 International Conference on Advance Computer Science and Information System*, pp. 347-352, 2011.

[15] L. Peng, Adaptive Median Filtering. Retrieve on 10 November 2015.

[16] S. Bhausaheb, M. Dnyandeo, P. Machindra, A.R. Dani, "Apply Different Filtering Techniques to Remove the Speckle Noise using Medical Images", *in International Journal of Engineering Research and Applications (IJERA)*, ISSN:2248-9622, vol. 2(1), pp. 1071-1079, 2012.

[17] A.S.A. Nasir, M.Y. Mashor, Z. Mohamed, "Modified Linear Contrast Stretching Algorithms: New Colour Contrast Enhancement Techniques for Microscopic Analysis of Malaria Slide Images", *Journal of Computational and Mathematical Methods in Medicine*, vol. 2012(2012), pp.1-17, 2012.

[18] L.Rosado, J.M.C.D. Costa, E. Dirk, S.C. Jaime, "A Review of Automatic Malaria Parasites Detection and Segmentation in Microscopic Images", *Journal in Anti-Infective Agents in Medicinal Chemistry*, vol.14 (1), pp. 11-22, 2016.

[19] S.R. Basha, P.K.K. Reddy, "Discrimination of Textures using Texton Patterns", *Global Journal of Computer Science and Technology: F Graphics & Vision*, vol.15(3), 2015.

[20] P. Finne, R. Finne, A. Auvinen, H. Juusela, J. Aro, L. Maattanen, M. Hakama, S. Rannikko, T.L.J Tammela, U-H. Stenman, "Predicting the outcome of Prostate Biopsy in Screen-Positive Men by a Multiplayer Perceptron Network", *Urology, ELSEVIER*, vol.56 (3), pp. 418-422, Sept. 2000.

[21] P. Kaler, "Study of Grayscale Image in Image Processing", *in International Journal on Recent and Innovation Trends in Computing and Communication (IJRITCC)*, ISSN:2321-8169, vol.4 (11), pp. 309-311, Nov. 2016.