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AUTOMATED CLASSIFICATION OF MALARIA PLASMODIA FROM THIN BLOOD SMEARS MICROPHOTOGRAPH

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ABSTRACT

Malaria is one of the malignant diseases which can be found in a tropical climate country like Indonesia. Automated malaria detection using host blood samples is an important approach to improve time efficiency for diagnosing the disease and giving the treatment as soon as possible. This automated detection uses a thin blood smears microphotograph which is stained using a Giemsa stain as input material. Furthermore the microphotograph is proceed using image processing algorithm which consists of image pre-processing, image thresholding using Otsu method, blood cell segmentation, suspected erythrocyte classification, and plasmodium classification. Two stages of classification are used in plasmodium classification by observing the size of object and the intensity supported by the Bayes classifier. The output from this program is expected to determine the plasmodium species of human plasmodium i.e. Plasmodium falciparum, Plasmodium malariae, Plasmodium viva, and Plasmodium ovale.

Keywords: malaria, thin blood smear, malaria classification, plasmodium classification, bayes classifier, otsu tresholding.

INTRODUCTION

Health is an important factor for human life. Ideally, increasing of world population must be supported by increasing health service. Therefore, technology is important to be used and to support human health quality. One of the example is developed through this research, which is Automated Malaria Detection. Malaria is a disease caused by a parasite called Plasmodium infected in human body through mosquito bites [1]. There are five types of *Plasmodium (P.)* which known to infect human body, namely Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale, and Plasmodium knowlesi but normally Plasmodium knowlesi infects animals. When human is infected by Malaria, Plasmodium will first invade human liver and go into bloodstream. There are four stages of Plasmodium life cycle in human blood stream, namely ring stage, trophozoite stage, schizont stage and gametocyte/sexual stage.

Reference [1] has stated that globally an estimated 3.3 billion people are at risk of being infected by malaria and 1.2 billion are at high risk (>1 in 1.000 chance of getting malaria in year). According to the latest estimates, 198 million cases of malaria occurred globally in 2013 (uncertainty range 124-283 million) and the disease led to 584.000 deaths (uncertainty range 367.000-755.000). In Indonesia, Malaria is found mostly in eastern part of Indonesia where there are lacks of medical experts. Detection of Malaria is highly depended on the skill to check the blood smear through a microscope. With this reason, automated malaria detection is needed to help diagnosis as the first step to prevent Malaria.

This research is conducted to detect whether a person, which is called a host, is infected by Malaria or not and to recognize type of Plasmodium that infects the host based on thin blood smears microphotographs and the

type of Malaria that infects the host. Detection of malaria will be conducted by using thin blood smears microphotographs which contain infected red blood cell (RBC) with Plasmodium. Therefore this research will contribute important input to reduce number of mortality due to Malaria.

RELATED WORK

Researches related to Malaria detection are previously conducted by several researchers based on thin blood smears microphotographs [2]-[6]. Anggraini et al. has proposed an automated Malaria detection based on thin blood smears microphotograph [2]. The established automated detection is focused on detecting P. falciparum in ring stage with dual Otsu thresholding method to convert input image into three levels intensity (black, grey, and white colour) image. Based on this image, chromatin dot on parasite's nucleus is identified.

Savkare S. S. and Narote S. P. has also built an automatic detection of Malaria by detecting the number of that appeared in thin blood microphotograph [3]. The research is used to classifiy whether an Red Blood Cell (RBC) is infected by Malaria or not by using Support Vector Machine (SVM) classifier based on geometry, colour, and statistical of the extracted features. In the research, type of Plasmodia is not mentioned and only one type of Plasmodia is used.

Díaz, González and Romero conducted a research on Malaria detection and stage of Plasmodium infection [4]. The Malaria detection is focused on *Plasmodium* falciparum and the classification is conducted based on stages of Plasmodium infection using Multilayer Perceptron Neuron (MLP) and Support Vector Machine Segmentation process conducted normalized RGB colour space and followed by Inclusion-Tree to separate pixels either as erythrocyte or

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background. In order to improve accuracy, clump splitting algorithm is used after segmentation process. Ahirwar, Pattnaik and Acharya [5], in their research on classification of malaria species, use SUSAN (Smallest Univalue Segment assimilating Nucleus) algorithm in image pre-processing combined with Granulometry algorithm to estimate the size of component in microphotograph. Four species of Plasmodia are also used as data sets in their research which classified using Back Propagation Feed Forward (BFF) neural network. BFF neural network architecture is chosen since it is simple and one of the most commonly used Artificial Neural Network

Using similar method with Ahirwar [5], Soni has done automated classification detection for *P.falciparum*, P.malariae, P vivax and P.ovale based on thin blood smears using SUSAN (Smallest Univalue Segment assimilating Nucleus) algorithm [6] combined with Granulometry technique to conduct image segmentation and estimate size for each component. Then huesaturation-intensity image size is used for classification. The purpose of this paper is similar with Soni [6] to detect P.falciparum, P.malariae, P vivax and P.ovale based on thin blood smears but using different approach, Image Processing Algorithm. It is constructed with image preprocessing, image thresholding, blood cell segmentation, suspected erythrocyte classification, and plasmodium classification. Two stages of classification are used in plasmodium classification by observing the size and intensity of the object supported by the Bayes classifier.

IMAGE DATA SETS

Datasets used in this research is thin blood smears microphotographs which acquired from Eijkman Institute for Molecular Biology Indonesia. Datasets contain Red Blood Cell (RBC) infected by P. falciparum, P. malariae, P. vivax and P. ovale. The sample images are captured using 5-megapixel Nikon digital sight DS 5Mc which is designed and built-in specifically for the light microscope, but in this research the images are generated using 1.2-megapixel resolution only with microscope's magnification 10 x 100. The images are saved in JPEG format in 1280 x 960 pixels size.

METHODOLOGY

There are five designed steps for Plasmodium classification which are image pre-processing, image segmentation, feature extraction, and classification. Figure-1 shows the flowchart of the overview designed system for automated Plasmodium classification.

As seen in Figure-1, the designed system is started by doing Image pre-processing techniques (convert image to greyscale image and noise removal), Image segmentation techniques (apply Otsu's thresholding method for converting image into binary image, apply hole filling algorithm, and connect component extraction), Feauture extraction, Suspected erythrocyte classification, and Plasmodia species classification.

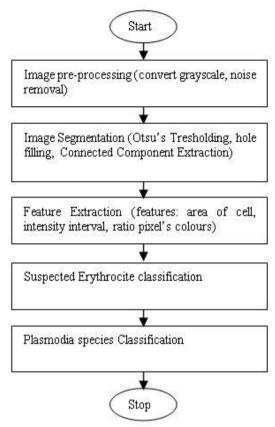


Figure-1. Flowchart of overview designed system.

Image pre-processing

The main goal of image pre-processing is to convert input image to greyscale image. When an image is written in .ppm format, the image is in RGB (Red Green Blue) colour composition. The input image later turns to be greyscale image so then can be used in further image processing step. After the image is converted, noise reduction technique is used. Median filter 5x5 is choosen to reduce noise in greyscale image in this research.

Image segmentation

When greyscale image is obtained, it is converted to be binary image by using Otsu's thresholding method [7]. The method is used to find threshold value. After threshold value is found, pixel intensity value will be categorize into 2 colours. If the pixel intensity value is below or equal to threshold value, pixel's intensity value is converted to be 0 (black colour) and if the pixel intensity value is more than threshold value, pixel's intensity value is converted to be 255 (white colour). Usually pixel with intensity value 0 is the objects and pixel with intensity value 255 is background. Example of Otsu's thresholding image is shown in Figure-2.

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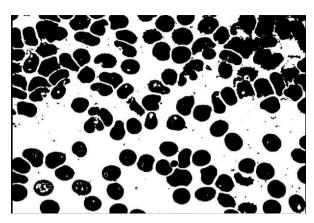


Figure-2. Example of binary image as resulted from Otsu's thresholding algorithm.

In Figure-2, black colour objects represent blood cells and noises and white colour is the background. Some of blood cells have holes in the middle due to biconcave charactheristics of RBC.

In binary image, blood components should be turned to be black as objects and blood plasma should turn to be white as background. Due to biconcave characteristic which made white areas appear in the middle of an object, hole filling algorithm is then used to fill the area in the middle. When hole filling is applied, each object is ready to be recognized as separated object using connected component extraction technique. This technique is used to extract the connected component become separated one. It gives different labels for every separated component in image. Separated components are separately analysed later for feature extraction for recognizing each of component.

Feature extraction

There are seven feature extractions used for classification. Some of features are extracted only to detect one or two types of Plasmodium while others are used for all four types of Plasmodium. In this study we use several features extracted from image including area of cell, intensity interval, ratio of three levels intensity, ratio of white region pixels around, ratio grey and black pixel before and after hole filling applied, number of black regions, shape factor for microscopic analysis, and statistical properties.

Area of cell and intensity interval

Different blood components has different size and colour which makes them unique to each other [8]. In order to determine type of blood component, area of cell and intensity interval are used. Area of cell and intensity interval determine whether a separated component is a healthy RBC, infected RBC, or other blood component. Area of cell is extracted by counting number of pixels constructed one separated component. Intensity interval is obtained by finding difference between maximum and minimum of intensity value in one separated component. Area of cell is also used later for Plasmodia classification as training sets in Bayes classifier since each of Plasmodium has different range of size [8].

Ratio of three levels intensity

Another extracted feature is the ratio of three levels intensity. This ratio represents the ratio of black, grey, and white pixels. Before this feature is extracted, a separated component is converted first based on grey level intensity values to three levels intensity (black, grey, and white) image using dual Otsu's thresholding [10]. Figure-3 shows example of component in greyscale and three levels intensity image.

This feature extraction is used to detect infected RBC by P.falciparum ring and P.malariae. When P.falciparum ring is exist in RBC, it has 1-2 chromatin dots and delicate cytoplasm [8]. When P.falciparum ring infected RBC is turned to three levels intensity image, the chromatin dots are turned into black pixels, cytoplasm of parasite is turned to white pixels, and cytoplasm of RBC is turned to grey pixel. These changes are used to detect the presence of P.falciparum as discussed in next section. When P.malariae is exist in RBC, it forms a basket or ribbon form with yelowish-brown pigment in trophozoite stage and it has several merozoites with velowish-brown pigment and cytoplasm which almost fill RBC's cytoplasm in schizont stage [8]. When infected RBC is converted to three levels intensity image, mostly merozoites are turned to be black pixels, parasite's cytoplasm is turned to be grey pixels, and RBC's cytoplasm is turned to be white pixels. These changes are used to detect the presence of P.malariae as discussed in next section. Figure-3 is two objects after being converted using dual Otsu's thresholding method. In the end, the composed colours of the object is only consisted of black, grey, and white colours.

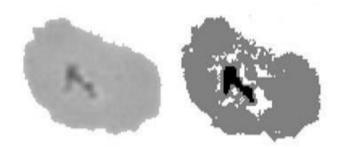


Figure-3. Example of greyscale object (left) and its three levels intensitiy object (right).

c) Ratio of white region pixels around

This feature is used to detect *P.falciparum* ring. One of unique biology fact regarding *P.falciparum* ring is chromatin dot which appear surrounded by parasite cytoplasm [8]. With this characteristic, infected RBC by P.falciparum ring is converted to three levels intensity images which will turn chromatin dot to black colours, RBC's cytoplasm to grey colour and parasite's cytoplasm to white colour. From this three levels intensity image, we calculate ratio between black region and white region surrounded the black region [2]. Figure-4 shows the example result of three levels intensity images from infected RBC with P.falciparum ring.

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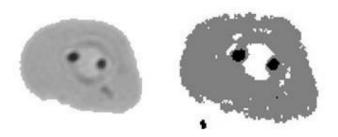


Figure-4. Infected RBC with *P.falciparum* ring in greyscale (left) and three levels intensity image (right).

With three levels intensity image, RBC's cytoplasm is converted to grey colour, vacuole of parasite is converted to white colour, and chromatin dot of parasite is converted to black colours. When an infected RBC is in three levels intensity form, computer can easily detect the presence of chromatin dot which indicated by black colours area and then the ratio of white colour pixels around chromatin dot. In the Figure-4, there are two chromatin dots in one parasite.

Ratio grey and black pixel before and after hole filling applied

Before this feature extracted, image must be converted first to three levels intensities image. This feature is extracted to detect P.malariae especially in trophozoite stage. When P.malariae is in trophozoite stage, parasite is in "band-form / basket form", has darkbrown colour and parasite is appear to be compact [8]. Those characteristics produce hole (white region pixels) in the middle of parasite after image is converted to three levels intensities image. This hole is then filled using hole filling algorithm and ratio of pixels before and after hole filling is counted. Figure-5 shows example of infected RBC with *P.malariae* in trophozoite stage.

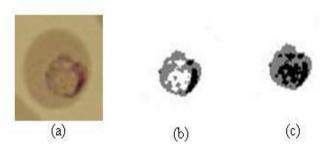


Figure-5. Infected RBC by P.malariae trophozoite in original image (a), three levels intensity image before hole filling (b) and after hole filling applied (c).

When image is converted from original colour into three levels intensity image, cytoplasm of RBC is turned into white which leaving the object looks smaller because only the parasite is exist. Parasite has a hole in the middle due to its "basket form" charactheristics. This hole is then filled using hole filling algorithm with black pixels. The number of black pixels that filled the middle hole is counted to detect whether P. malariae is exist or not.

Number of black regions

Image must be converted first to three levels intensity image. This feature is used to detect *P.malariae* especially in schizont stage. When P. malariae is in schizont stage, merozoites are appeared inside infected RBC which makes some black regions appeared in three levels intensity image [8]. Number of black regions is calculated. Figure-6 shows example of infected RBC with P. malariae in schizont stage.





Figure-6. Infected RBC with *P.malariae* schizont in greyscale image (left) and three levels intensity image (right).

As seen in Figure-6, P. malariae schizont is indicated with presence of merozoites which form several black coloured dots. From the three levels intensity image, we can see clearly several separated black regions which indicated the merozoites. This black regions are then counted to know whether RBC is infected by P. malariae schizont or not.

Shape factor for microscopic analysis

Several shape factor analysis used in this research are circularity, compactness which is based on perimeter and area of cell. While area of cell is found by counting all pixels in one component, perimeter is calculated by counting pixels in one component whose one or more neighbour pixel which is not belong to one component. If area of cell is denoted with A and perimeter is denote with P, Equation. (1) shows rule to calculate Circularity and Equation. (2) shows rule to calculate Compactness.

$$Circularity = \frac{4\pi A}{P^2} \tag{1}$$

$$Compactness = \frac{P^2}{A}$$
 (2)

Circularity and compactness are used to distinguish between infected RBC by P.vivax or P.ovale and overlapping cells. These treatments are applied because infected RBC by P.vivax or P.ovale tend to have enlarged size of RBC [8] which has area of cell that is almost same with overlapped cells.

g) Statistical properties

There are some statistical rules used in this research for detecting infected RBC by P.vivax or P.ovale in trophozoite and schizont stage. When RBC is infected by P.vivax, RBC will be enlarged maximum twice from averaged normal RBC's size. While when RBC is infected with P.ovale, RBC will be enlarged maximum 1.25 x

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normal size of RBC [8]. In trophozoite stage, P.vivax has large ameboid cytoplasm, large chromatin and velowishbrown pigment while in schizont stage, P.vivax is almost fill RBC with mature 12 - 24 merozoites and fine yelowish-brown pigment [8]. In schizont stage, P.ovale has compact cytoplasm with large chromatin and coarse dark-brown pigment while in schizont stage, P.ovale is almost fill RBC with mature 6 – 14 merozoites with large nuclei and dark-brown pigment [8].

Data used for statistic rules is pixel intensity value which composed one component. Based on intensity values, this research count mean, standard deviation, skewness, kurtosis and entropy for each suspected component. The results are later used to generate classification rules. Equation. (3)-(7) shows statistical rules used in this research with x denotes as intensity value in greyscale, P(x) denotes as probability of intensity x occurred in whole image and greyscale image has range from 0 as the minimum intensity value until L as the maximum intensity value.

$$Mean = \bar{x} = \sum_{0}^{L-1} x P(x)$$
(3)

Standard Deviation =
$$S_d = (\sum_{0}^{L-1} (x - \bar{x})^2 P(x))^{\frac{1}{2}}$$
(4)

Skewness =
$$\frac{1}{S_d^3} \times \sum_{0}^{L-1} (x - \bar{x})^3 P(x)$$
 (5)

$$Kurtosis = \frac{1}{S_d^4} \times \sum_{0}^{L-1} (x - \bar{x})^4 P(x) - 3$$
(6)

$$Entropy = -\sum_{0}^{L-1} P(x) log_2 P(x)$$
(7)

Classification

In this research, there are 150 thin blood smears microphotographs as datasets. Datasets consist of mixed images which are infected by four types of Plasmodia. Datasets are then divided into training sets and testing sets with ratio 1:1 for training sets and testing sets. Training sets will be used for generating classification rule and testing sets to measure accuracy of the system.

Based on the observation, an infected RBC has area of cell between 3.000 and 11.000 pixels and intensity interval which is more than threshold value. Threshold value for intensity interval is determined by normal distribution rule where there are 68% values resulted from one strandard deviation away from mean and it draws 95% values when there are standard deviation are two. Threshold value is determined by taking limit value in 1,5 of standard deviation away from mean. Suppose X is the threshold value, σ and μ is standard deviation and mean from intensity values in one connected component. Equation. (8) shows the rule to get threshold value used to detect infected erythrocyte.

$$X = \mu + 1.5 \sigma \tag{8}$$

After a component identified as infected by Malaria, system will detect the type of Plasmodium. There are 2 stages for Plasmodium classification. At the first stage, size of infected RBC is estimated whether it is enlarged or not using Bayes classifier in order to minimize possibility type of Plasmodium infected. When infected RBC is identified not enlarged, infected RBC is possibly infected by P.falciparum or P.malariae and when infected RBC is identified not enlarged, infected RBC is possibly infected by P.vivax or P.ovale [8].

When an RBC is infected by P.falciparum ring, first classification rule is to detect when a component is turned to three levels intensity image, ratio of black pixels should be very small, and ratio of grey pixel should be dominated. When it is passed first classification rule, ratio of white pixels surround black pixels region should be calculated and the result should be around 1 if it is infected by P.falciparum ring.

If RBC is infected by *P.malariae* trophozoite, after component is turned to three levels intensity image, ratio of white pixels should be bigger than ratio of grey pixels and ratio of grey pixels should be bigger than ratio of black pixels. When it is true, hole filling is applied in the component and ratio of pixels before and after hole filling should be equal or more than 1,1.

If RBC is infected by P.malariae schizont, after component is turned to three levels intensity image, ratio of grey pixels should be dominated due to parasite cytoplasm which covers almost cytoplasm of RBC. After that, ratio of black pixels should be bigger than ratio of white pixels since black pixels symbolized the present of merozoites. When it is true, number of black region is counted. When RBC is infected by P.malariae schizont, number of black component should be more than 3.

For detecting P.vivax and P.ovale, there are two steps should be conducted after Bayes classifier applied. First step, this research uses circularity, compactness, and standard deviation as limit to filter whether infected RBC is really infected RBC or others blood component which may lead to false positive. If component is really an infected RBC either by P.vivax or P.ovale, circularity must be more than 0.45, compactness must be more than 19, and standard deviation must be more than 13. Second step, to detect whether infected RBC is infected by P.vivax or *P.ovale*, statistical rules are used in training sets. When it is done, Weka software [9] is used in this research based on result from training sets. Results are converted to .arff file and then using JK48 tree node classifier it is classified to generate decision tree. Figure-7 shows decision tree to determine whether infected RBC is infected by P.vivax or P.ovale.

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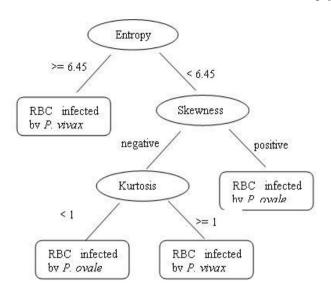


Figure-7. Decision tree for classification of *P.vivax* and P.ovale.

Entropy is used to indicate how random the intensity values contructed one component. If object has likely same intensity values, entropy is more likely to be low. Skewness is used to know whether the object is more likely has high value or low value of intensity. Skewness is negative if high value of intensities are dominant and positive if low values of intensities are dominant. Kurtosis measures peakedness of intensity values in one object. If kurtosis is equal or more than 1, a single or range of pixels are dominated in one component.

At the end of this research, accuracy of the automated detection is measured by calculating sensitivity, precision first [10]. The way to calculate sensitivity is shown in Equation. (9), the way to calculate precision is shown in Equation. (10) and the way to calculate accuracy is shown in Equation. (11).

$$Sensitivity = \frac{TP}{TP + FN} \tag{9}$$

$$Precision = \frac{TP}{TP + FP} \tag{10}$$

$$Accuracy = \frac{2 \times Precision \times Sensitivity}{Precision + Sensitivity}$$
(11)

Based on Equation. (9)-(11), TP is total number of True Positive, FN is total number of False Negative and FP is total number of False Positive.

EXPERIMENTAL RESULT AND DISCUSSION

Based on the features gathered and classification rules generated, testing sets are tested and accuracy rate is counted. Testing sets are mixed consist P.falciparum, microphotograph which infected by P.malariae, P.vivax, or P.ovale.

Suspected erythrocyte classification result a)

accuracy of suspected erythrocyte classification for healthy RBC and infected RBC detection are measured with sensitivity, precision, and accuracy as stated before in Equation. (9)-(11). In healthy RBC detection, there are 9.010 true positive cases, 22 false positive cases, and 33 false negative cases which lead to sensitivity at 99,6%, precision at 99,8%, and accuracy at 72%. For infected RBC detection, there are 94 true positive cases, 50 false positive cases, and 35 false negative cases which lead to sensitivity at 73%, precision at 65%, and accuracy at 69%.

Plasmodium falciparum classification result

Based on classification rule determined to detect P.falciparum ring, 26 components infected P.falciparum ring are used as testing set. Based on the experiment, 18 components are successfully identified as infected by P.falciparum ring and 8 components are false negative. Furthermore when whole microphotographs is tested, it is found there are 6 images miss identified as infected by P.falciparum ring which lead to false positive for P.falciparum detection. Sensitivity, Precision, and Accuracy for P.falciparum detection are calculated based on Equation. (9)-(11). At the end of calculation, Sensitivity is 69%, Precision is 75%, and Accuracy is 72% for P. falciparum detection.

c) Plasmodium malariae classification result

Based on classification rule determined to detect P.malariae, 11 components infected by P.malariae are used as testing set. Based on the experiment, 8 components are successfully identified as infected by P. malariae, 3 components are false negative. Furthermore when whole microphotographs is tested, it is found there are 3 images miss identified as infected by P. malariae which lead to false positive for P. malariae detection. At the end, sensitivity, precision, and accuracy for *P.malariae* are calculated based on Equation. (9)-(11). Sensitivity for P.malariae detection is 73%, precision for P. malariae detection is 73%, and accuracy for P.malariae detection is 73%.

d) Plasmodium vivax classification result

Based on classification rule determined to detect P.vivax, 12 components which infected by P. vivax are used as testing set. Based on the experiment, 8 components are successfully identified as infected by P. vivax, 4 components are false negative. Furthermore when whole microphotographs is tested, it is found there are 3 images miss identified as infected by P.vivax which lead to false positive for P. vivax detection. At the end, sensitivity, precision, and accuracy for P.vivax are calculated based on Equation. (9)-(11). Sensitivity for P.vivax detection is 67%, precision for P. vivax detection is 73%, and accuracy for *P.vivax* detection is 70%.

Plasmodium ovale classification result

Based on classification rule determined to detect P.ovale, 14 components which infected by P. ovale are used as testing set. Based on the experiment, 11 components are successfully identified as infected by P. ovale, 3 components are false negative. Furthermore when whole microphotographs is tested, it is found there are 3

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images miss identified as infected by P. ovale which lead to false positive for P. ovale detection. At the end. sensitivity, precision and accuracy for P.ovale are calculated based on Equation. (9)-(11). Sensitivity for P.ovale detection is 79%, precision is 79%, and accuracy is 79%.

The summary result of the experimentations can be seen in Table-1.

Table-1. Experimental result.

No.	Plasmodium Type	Sensivity	Precisio n	Accuracy
1	Healthy RBC	99.6%	99.8%	72%
2	Infected RBC	73%	65%	69%
3	P. Falciparum	69%	75%	72%
4	P. Malariae	73%	73%	73%
5	P. Vivax	67%	73%	70%
6	P. Ovale	79%	79%	79%

CONCLUSIONS

In order to classify Plasmodium species, an input must pass image pre-processing, segmentation, feature extraction, and classification. From the result, we conclude that the purposed methods are able to identify infected RBC by P.falciparum, P.malariae, P.vivax, and P.ovale

For P.falciparum, only ring stage is able to be detected in this program. For P.malariae, P.vivax, and P.ovale, the program is able to detect at trophozoite and schizont stage. The accuracy (F1) of the program to correctly classify is 99,8% for healthy RBC detection, 69% for infected RBC detection, 72% for P.falciparum ring detection, 73% for P.malariae detection, 70% for P.vivax detection, and 79% for P.ovale detection.

The future work of the study are addressed to the following issues:

- Use larger datasets on the study in order to know further classification result.
- Improve the classification of each Plasmodium in other stages of Plasmodium to cover more possibility of infected erythrocyte.
- Feature that able to separate overlapping cells is added in order to analyze overlapped cells.

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