Plasmodium Parasite Detection on Thin Blood Smear Image using Double Thresholding and BLOB Analysis

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***Abstract*—Detection of the parasite that causes malaria can be made through examination of blood slides observation using a microscope by medical officer. However, to prevent death in people with urgently needed malaria parasite detection can be done rapidly and accurately, mainly in such areas which is lack of experienced medical officer, such as in rural areas in Borneo and Papua. Furthermore, in an effort to malaria diagnosis, the data are inconsistently made with standard procedure, hence the blood smear data produced are non-standard data. This becoming a huge challenge for the researcher to be able to detect parasite on non-standard data. By using microscope, eyepiece camera, and computer, we propose a method to detect the presence of parasite inside the red blood cells using combination of the Double Thresholding techniques and BLOB analysis. Our experimental research using our proposed method, able to obtain average of PPV of 84.43%, and Sensitivity of 85.5%.**

Keywords — BLOB Analysis, Double Thresholding, Image Processing, Malaria, Plasmodium Detection.

I. INTRODUCTION

Malaria is one of kind of a disease that caused by plasmodium parasite. The parasite transmitted into human body through the bite of infected female anopheles mosquito [1]. Malaria can cause death in patients [2], especially in group of infants, toddlers, and pregnant women. Based on World Malaria Report 2016 [3], in 2015, estimated around 212 milion cases of malaria occured worldwide. In Indonesia, cases of malaria in 2013 increased by twice compared to the case in 2010 [4]. Based on data from the Ministry of Health Republic of Indonesia in 2016 [1], the highest malaria cases are in Papua which was followed by the region of East Nusa Tenggara, Maluku, Bengkulu, and Kalimantan (Borneo Island).

Plasmodium parasites that infect human blood will grow and multiply inside the red blood cells (RBC). The parasite will destruct the RBC, infect others RBC and so on. In meantime, the number of healthy RBC will decrease and can cause severe damage into human body that will lead into death. Severe malaria commonly manifests with one or more of following condition such as coma (in case of parasite attack the brain cells known as cerebral malaria), severe anemia, hypoglycaemia, metabolic acidosis, acute pulmonary oedema, or acute renal failure, which if left untreated or delayed treated, severe malaria is lead to a fatal disease cases [5].

According to its fatal risk and high number of cases found worldwide, we need a fast and accurate detection method that can help the medical officer to detect Plasmodium parasite in human RBC. The Plasmodium parasite presence detection technique that has been widely used in a routine check is to use a microscope [6] [7]. Plasmodium parasite presence in blood cells was easily detected by an expert using microscope. However, the disadvantage of this monitoring technique using microscope is highly dependent on the existence and competence of medical experts. The accuracy of the examination using microscopic can be decreased 64% up to 95% as the number of parasites in the RBC decreases, or else in the case of mixed infections with only one parasite detected [8].

Another techniques to detect the presence of Plasmodium parasites is to use Rapid Diagnostic Test (RDT) which is sold commercially. However, RDT is not capable of detecting all types of Plasmodium, mostly just that type of Plasmodium falciparum [9].

A standard detection of parasites using a microscope to identify the life cycle state of parasite takes time, and the diagnosis depend on the expertise and their experience [8] [10], and in some areas in Indonesia lack of experienced medical staff. The example of infected RBC from our data shown in fig. 1 below:

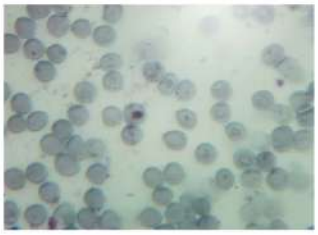

Figure 1. RBC infected by parasite.

II. RELATED WORK

In medical field, in order to accurately diagnose malaria disease, they use laboratory blood examination technique such as microscope and Rapid Diagnostic Test (RDT). The detection of Plasmodium presence using microscope [11] [12] [13] is the most widely used because in addition can detect parasite species, it can also be determined the number of parasites and the progress of treatment [6] [7]. Microscope is most reliable in the hands of medical experts but less sensitive and precise in routine inspection activities. This can be caused by errors in the process of collecting, processing or examinating the blood slides, interpretating, and reporting. The errors occur depending on the competency of the laboratory technicians, physical limitations in the workplace, tools quality, microscopes and workload conditions [9].

Another tool to detect malaria parasite, we can use Rapid Diagnostic Test (RDT). RDT can be used in places with

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limited resources and facilities. The results showed that the RDT is more sensitive for detecting plasmodium falciparum but less for other parasites. RDT sensitivity for detecting infection by non-falciparum parasite is only reach 50-52%, hence the microscope is still the best option tool [14].

The gold standard for diagnose the presence of parasite in human RBC is constantly depend on the use of Microscopy technique [15], but the lack of experienced medical officer is a major obstacle in efforts to reduce mortality numbers caused by malaria. According to the results of our in-situ surveys in the area of Central Borneo, some areas still require atleast three days to get the results of laboratory tests.

Lack of medical officer is one of the prohibitive factors in reducing the number of deaths caused by malaria. In this case, many researchers proposed theirs methods in attempt to detect parasite automatically. One of the studies that have been carried by Gatc et al [2], in order to detect the presence of parasites in the RBC, Gatc et al. propose double Thresholding method. Gatc et al. research obtain PPV of 92.85%, and sensitivity of 85.52%.

Arco et. al. [16] use morphological approach in order to analyze malaria parasites. Sulistyawati et al. [17] propose BLOB analysis on thick blood films image in order to segregate parasite. Sulistyawati et al research obtain avera accuracy of 99.392%. In addition, Kareem [18] proposed A Hybrid Illumination and Color Constancy insensitive morphological Approach can obtain accuracy of 95.7%.

Nanoti [19] propose KNN classifier to train 300 images data from CDC and WHO. Nanoti proposed method obtain accuracy of 90.17% and sensitivity of 90.23% in order to detect three lifecycle stages of the Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale. These life cycle stages are: trophozite, schizont, and gametocyte for each species. Bibin [20] propose Deep Believe Networks to classify about four thousand peripheral data in binary class which is parasite or non-parasite class. Bibin achieve evaluation score: sensitivity value: 97.60%, F-score value: 89.66%, and specificity value: 95.92%.

Devi [21] propose classification system based on the histogram feature set using Artificial Neural Networks, k-Nearest Neighbor, Support Vector Machine, and Naive Bayes classifier on erythrocyte data. Devi proposed method obtain accuracy and F-score: 96.32% and of 85.32% respectively. Srivastava et. al. [22] using PCR to analyze samples from 431 consented patients. Srivastava et. al. proposed method able to classify of the falciparum and vivax positive samples of 73.3 % and 91.4 % respectively. On our previous study, we proposed a method to distinguish the WBC from RBC [26] due to similarity of the infected RBC and WBC appereance.

In line with lacking medical officer, blood slides making procedures must be standardized in order to produce good quality slides data thus simplifying the process of detection of the parasite. In fact, not all data slides are made meet standard procedures. According to our in-situ survey in Central Borneo, many of blood slides produced are non-standard due to the lack of experienced medical staff. Non-standard data may be the big obstacle in the detection of the parasite. The examples of standard and non-standard data can be seen in fig. 2.

Non-standard data could be that too little amount of blood smear applied on the slide hence the blood sample (fig. 2.a.) will have the probability of parasites absency, while the too much amount of blood smears slide (fig. 2.b.) make it difficult to observe because the cells are stacked on each other. Most of studies related to the detection of the parasite are using standard data, hence the detection of the parasite is easily done. The detection of parasite requires many researches development and improvement techniques when it come to the fact that the data produced in many areas which is lack of experienced medical officer are non-standard data as seen in fig. 2.a and 2.b.

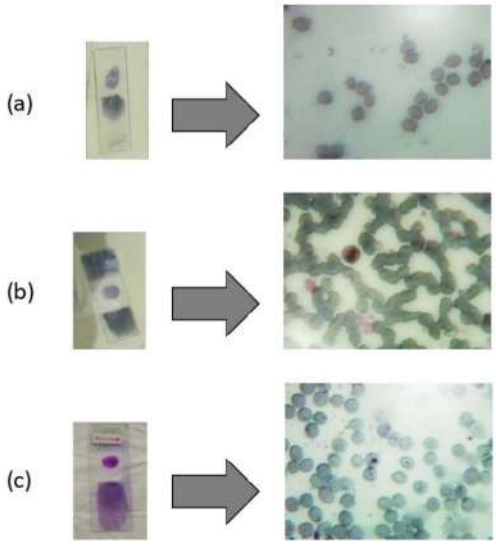


Figure 2. (a) and (b): Non-Standard slide data; (c)Standard slide data

III. METHODOLOGY

The workflow of this study shown in fig. 3:

Figure 3. Research workflow

A. Data

The data use in this study taken from the same resource of our previous study in 2016 (see Gatc [26]) with permission granted by Department of Health in Central Borneo Province Republic of Indonesia and validated by the Manager of Microbiology Engineer in Laboratory of Health in Palangkaraya Indonesia. The data exclude the patient's personal data (such as name, age, address, personal photo, etc). We use 20 thin blood positive malaria data. The thin blood smears data are made by medical workers who are less experienced with Giemsa standard techniques [23] [24].

Image data obtained by putting the slide data under the microscope objective lens. The image data captured using digital eyepiece camera with 2MP resolution attached to microscope as it ocular lens, and connected to a computer with Universal Serial Bus (USB) cable. The data captured in RGB format and jpeg files type.

B. Method

In this research, we use combination technique of Double thresholding method [2], Blob Analysis [18] [25] [26] [27], and morphological approach [16] [17] with aim to improve detection evaluation result. Our proposed method steps are:

a. Pre-processing

At this step, the first thing to do is convert the image from RGB to grayscale in order to reduce computational complexity. Afterwards, we use 3x3 windowing median filters to reduce noise.

b. Double Thresholding-First Thresholding

The main idea of first thresholding is to seperate foreground (blood cells) from its background. The main steps of this process can be found in our previous study (see Gatc et al. research in 2013 [2]).

c. Morphological Method

On step (b) above, we distinguish foreground from the background, in this case, the foreground is blood cell that shaped like disks in the image. Thresholding divides the two regions based on gray-level values in an image into a binary image (0 and 1). In fact, the blood cells have low intensity at the center of the cell. After thresholding method process, the central part of the blood cells will be considered as background. Hence the cells object will have a hole inside the cell (similar to donout shape). Inspite to detect parasite, we have to take the cell object as a whole cell object. We use fill-holes morphological techniques to solve these cells holes problem.

Fill holes is one of image morphology techniques for the representation and description of an object in the image data such as skeletons, boundaries, and the convex hull. Region filling is based on a number of dilation, complementation, and intersection. Region Filling Process (fill holes) aim to fill entire area with a value of 1. Fill holes formula can be seen below:

$$X_i = (X_{i-1} \oplus B) \cap A^c \quad i = 1, 2, 3, \ldots \qquad (1)$$

where:

- X_0 is forming an array of zeros with the size of array A, besides, the X_0 is set to value of 1.
- B is the symmetric structure element.
- The algorithm terminates at the iteration step i if $X_i = X_{i-1}$.
- The set X_i contains all the filled holes; the union of X_i and A contains all the filled holes and its boundaries.
- A contains all the filled holes and its boundaries [27] [28] [29].

The union set of X and A result will fill the filling region and its boundary. The fill holes technique results shown in fig. 4. After finding information about blood cells (foreground) pixels coordinates, the background information is no longer needed, so we set all the background pixels value into zero (0).

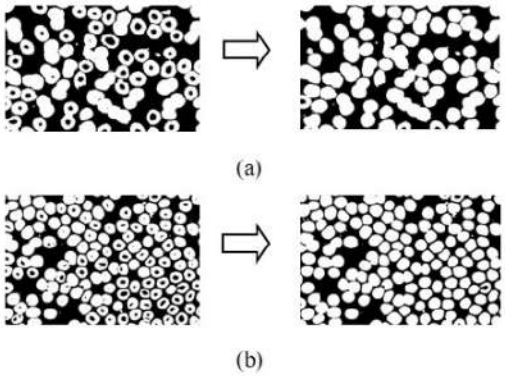
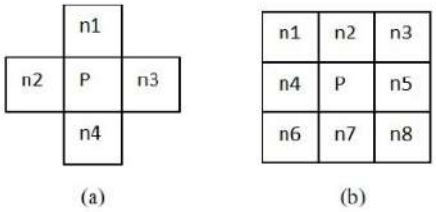


Figure 4. (a) and (b) Fill holes technique result

d. Features Extraction-Binary Large Object (BLOB) Analysis

BLOB can be refered to a group of pixels in a binary image that directly connected one to another pixels. The examples of BLOB shown in fig. 5 below:



(a) (b)

Figure 5. Blob pixels neigborhood; (a) 4 neigbors, and (b) 8 neigbors

BLOB defines "Large" in term of only certain size objects are considered as the interest region and the rest labelled as noise [27]. We use Recursive Grass-Fire Algorithm [30] as our searching method in aim to find BLOB. BLOB analysis [25] algorithm can be seen below:

(1) Identify individual blob by seeing which pixels are connected to each other.

(2) Each group of connected pixels will be given a label, a number as identifier to distinguish one blob from another.

(3) Do bwlabel() or bwconncomp() function on Matlab to each blob.

(4) Blobs labeled

e. WBC and RBC Identification

After features extraction process, we can distinguish WBC from RBC. We use binary image data, where nonzero pixel belong to an object and 0 pixels constitute the background. We use *Region Props* function to compute the binary image's object properties. We trace object's region boundaries and the exterior boundaries. We also trace the boundaries of holes

inside the objects. bwboundaries() descends into the outermost objects and then the enclosed objects.

In Matlab, we use black and white boundary computation function and this function will returns cells array, where each cell consist of coordinates for an object and also the number of cells. We compute and set feature of area less than 1500 pixels and large than 250 pixels. The extraction process restricted to blobs that meet our criteria and eliminate the rest. For each blobs that meet our criteria, will be labeled. Then we compute the *Intensity, Area, Diameter, Coordinate x, Coordinate y* all the labelled blob. Sample of features extraction result can shown in Table I below:

TABLE I. THE EXAMPLE OF FEATURE EXTRACTION RESULT FROM ONE DATA

Candidate	Intensity	Area	Diameter	Location X	Location Y
1	181	1	1.128379 167	1	1
2	**77.49094 047**	**4636**	**76.82928 172**	**39.69974 116**	**579.9199 741**
3	103.5	2	1.595769 122	1	603.5
4	168	1	1.128379 167	1	1498
5	96.47368 421	38	6.955796 338	446.3421 053	797.0789 474
6	81.62688 615	729	30.46623 751	514.9615 912	320.5089 163
7	**84.25073 008**	**2397**	**55.24450 37**	**607.3525 24**	**552.1422 612**
8	**90.66666 667**	**21543**	**165.6182 342**	**1246.499 977**	**858.3912 64**
9	**79.40285 205**	**2244**	**53.45231 088**	**1288.308 378**	**702.1430 481**
10	94.05010 893	459	24.17471 719	1725.217 865	1294.400 871
11	95.55470	393	22.36924	1755.765	1336.340
12	97	7	2.985410	1748	1313
13	98.33333	3	1.954410	1753.666	1314.666
14	98.16666	6	2.763953	1758.833	1361.166
15	**66.77131**	**11304**	**119.9695**	**2072.624**	**177.4764**
16	165	1	1.128379	2250	1
17	154	1	1.128379	2250	1498

We use all the features as seen in Tabel 1 to determine the difference pattern between RBC and WBC. According to Kareem [16], the average size of WBC is larger than RBC. Refer to Kareem, we use *area* feature to measure the expansion of cell area.

From Table I, we can see some candidates of blood cells such as cell number: 2, 7, 8, 9 and 15 that we considered as WBC. It can be seen from it greater area compared to other candidate area. We also record all the X and Y coordinate pixels of each extracted cell candidate. Refer to morphological approach used in this study, all WBC information from the image will be set to zero (0) and focus on to the infected RBC.

f. Parasite Identification - Second Thresholding

The main idea of this second thresholding is to seperate infected and normal (healthy) RBC. We use grey level intensity and area ratio features as described below:

(1) Set RBC candidates image to grayscale.

(2) Use Thresholding method to segment RBC with parasite, and normal RBC.

(3) Check the *intensity* and *area ratio* features.

(4) Labeling each infected RBC.

g. Evaluation

Our evaluation method formulas are shown below:

$$PPV = \frac{TP}{TP+FP} \quad (2)$$

$$Sensitivity = \frac{TP}{TP+FN} \quad (3)$$

where :

PPV is the positive test proportion that are classified as true positives, in this case, parasites classified as parasites hence represent the true presence of the parasite. Sensitivity value described the ability of our proposed method to classify the parasites in parasites or the true presence of parasites inside the RBC. TP is the number of parasites classified as parasite. FP is the number of non-parasites classified as parasites. While FN is the number of parasites classified as non-parasites.

IV. RESULTS

In this research, parasite detection evaluation using 20 data shown a good result. It can be seen from the PPV and sensitivity obtained. We visualize the experiments results in order to do some cross-check done by laboratory staff. The image of detection results that will be cross-checked by the laboratory staff can be seen in fig.6 below:

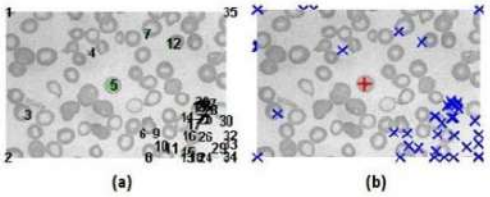


Figure 6. Detection result (a) Candidates of Parasite; (b) Parasites Detection

As shown in fig. 6, at the early detection in one of the data we use; it shows 35 parasite candidates. However, after the more specific calculation, our proposed method only gets one candidate that meets all the infected RBC criteria. In the visualization process, we use the "+" red mark to indicate the presence of parasites while the "x" blue mark to indicate another cell which is considered as a candidate parasite. We also visualize the entire candidates from one data image as shown in fig. 7 below:

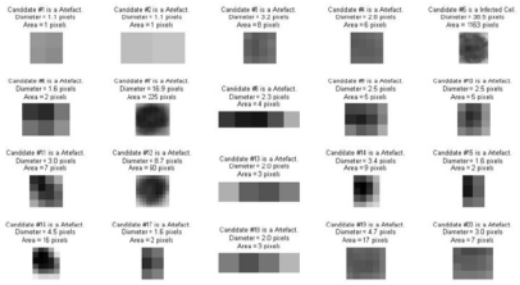


Figure 7. The candidates of parasite from one of experiment data

In Table II, can be seen PPV and Sensitivity value of our proposed method for each data. The average of PPV and Sensitivity value obtained from our study are 84.43% and 85.5% respectively.

TABLE II. EVALUATION RESULT

No.	Data	PPV	Sensitivity
1	data1	90.5%	89.4%
2	data2	87.1%	88.2%
3	data3	88.4%	85.1%
4	data4	87%	85.3%
5	data5	87.6%	88.7%
6	data6	85.1%	82.6%
7	data7	87.5%	84.2%
8	data8	84.4%	86.1%
9	data9	88.2%	87.2%
10	data10	84.7%	82.1%
11	data11	82.1%	88.4%
12	data12	80.2%	87.2%
13	data13	78.4%	81.3%
14	data14	81.4%	85.2%
15	data15	82.8%	85.7%
16	data16	87%	86.5%
17	data17	86.3%	86%
18	data18	81.2%	85.3%
19	data19	80.6%	84.5%
20	data20	78.1%	81%
	Average	**84.43%**	**85.5%**

CONCLUSION

In a lack of expert medical officer areas such as rural areas in Borneo and Papua, can be the major factor inhibiting in suspected malaria patient treatment. The less expert medical officer tends to produce non-standard blood smear, unable to detect any parasite in blood smear, hence misdiagnose suspected malaria patient. Non-standard data is a major problem for the researcher in order to develop automatic parasite detection especially in area that lack of experienced medical officer. In aim to deal with non-standard blood smear data, our proposed method achieves an average of PPV value of 84.43% and Sensitivity value of 85.5%. For future work, it is considered to do research throughout the parasite life cycle in order to effectively treat individual patient.

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