ANALYSIS OF MICROSCOPIC BLOOD SAMPLES FOR DETECTING MALARIA

SAW HUI ANN

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ABSTRACT

Malaria is a mosquito-borne disease and it has been affecting millions of people worldwide since decades ago. The conventional method in diagnosing the blood disease is by using manual visual examination of microscopy blood smears. However, a computer-assisted system can be designed to assist in malaria diagnosis by employing image processing, analysis and feature recognition algorithm. In terms of enhancing image for analysis, this study explores on a new approach by averaging results of two filters. In order to evaluate the performance of the proposed method, the image was further clustered by using K-Means, Expectation Maximization (EM) and Otsu's threshold algorithm .

ABSTRAK

Malaria merupakan sejenis penyakit yang dibawa oleh nyamuk dan ia telah mempengaruhi berjuta-juta insan di seluruh dunia sejak dekad dahulu. Cara konvensional dalam mendiagnosis penyakit tersebut adalah dengan pemeriksaan visual mikroskopik selaput filem nipis darah. Namun, sesuatu sistem bantuan komputer boleh direka dengan menggunakan algoritma pemprosesan imej, analisis dan pengecaman ciri untuk membantu diagnosis malaria . Dari segi pembaikan imej untuk analisis, kajian ini menerokai satu pendekatan baru dengan pengiraan purata hasil dua penapis imej . Dalam usaha untuk menilai prestasi pendekatan yang dicadangkan, imej tersebut telah dikelompokkan dengan menggunakan algoritma K- Means, Expectation Maximization (EM) dan kaedah Otsu.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Malaria affects millions of people worldwide.It remains as a significant public health problem in many tropical areas, especially in Africa. It is a parasitic disease caused by the protozoa of *Plasmodium* species. It can infect on both animals and humans. There are four common species of *Plasmodium* parasitic protozoan that infect humans, which are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. An occasional infection of monkey malaria parasites, *P.knowlesi* also happens . *P. falciparum* is the most severe type of malaria and it caused most of the fatality among the reported cases of Malaria. Malaria parasite is transmitted through *Anopheles* mosquitoes. Both a human host and an insect host are needed for malaria parasite to complete its life cycle which includes three stages, *exo-erythrocytic stage* in liver cell, *erythrocytic schizogeny stage* in red blood cell and *sexual stage* in which the gametocytes develop in the insect host (Crutcher JM., 1996).

When an infected *Anopheles* mosquito bites a person, the *sporozoites* (the protozoa which develops in the midgut of anopheline mosquito and migrate to the salivary gland) may be injected along with the saliva into human blood vessels. Within 30 minutes, some of the sporozoites are destroyed by the immune system cells, but some will enter liver parenchymal cells. In the liver cells, the parasites multiply into a liver-stage *schizont* which contains 2000 to 40,000 of *merozoites*. This amplification process is called exo-erythrocytic schizogony. Among four of the malaria parasite species that affect humans, there are two species that hava a slightly different cycle during liver phase, which are *P. vivax* and *P. ovale.* The sporozoites that enter the liver

cell do not immediately mature into schizonts, but instead becomes a *hypnozoites*, which can lay dormant in the liver cell and induce relapse of infection that occur at a later date.

Regardless of the development time of the schizonts, the mature schizonts will eventually rupture and releases thousands of *merozoites* into the bloodstream and infect the erythrocytes (red blood cells) . This marks the start of erythrocytic schizogeny stage. Each merozoite will infect each erythrocyte. The merozoite that entering an erythrocyte will starts to reproduce asexually and transforming into *trophozoite* . The trophozoite will then divides gradually and mature to schizont. Eventually the infected erythrocyte ruptures and releases new merozoites, which will continue to infect more erythrocytes. However, during erythrocytic schizogeny phase, instead of forming trophozoites, some of the merozoites will develop into immature gametocytes (Fishman & Fishman, 2006) .

The gametocytes are stimulated and mature to *microgamete* (Figure and *microgamete* (female) in the guts of female anopheles mosquito after the mosquito ingested the human blood containing gametocytes. The microgamete and macrogamete will then reproduce sexually and this starts the third stage of plasmodium life cycle. Sporozoites will be the product of this stage and another cycle will begins when the anopheles mosquito ingests blood of a new human host.

According to the latest world health statistics by World Health Organization (WHO), Malaysia was listed as pre-elimination country with 5306 reported cases in Malaysia in 2011. Even though the situation of Malaria infection in Peninsular Malaysia is under controlled , but Sarawak and Sabah are still being found as the highest endemic area in Malaysia (World Health Organization, 2012). Hence, early diagnosis and effective treatment is the key factor to reduce the fatality rate of malaria infection. The diagnosis of malaria disease is done based on presumptive analysis on infection symptoms , followed by clinical diagnosis supported by the detection of parasites in the blood (parasitological or laboratory diagnosis) and additional haemotological examinations.

The laboratory diagnosis by microscopic examination of malaria based on Giemsa-stained blood smears remain as the common reference standard or the chosen procedure for the detection and identification of *Plasmodium* parasites. There are two kinds of blood smears that are used for microscopic examination of malaria parasites, which are thick blood smears and thin blood smears. Thick blood smears are best for establishing the presence of *Plasmodium* parasites while thin smears aid in species identification (Shetty, Tang, & Andrews, 2009). Both thick and thin blood smears should be prepared and used for morphological identification of malaria parasites (Houwen, 2002).

Principally, blood smears are prepared by placing a drop of blood on a clean glass slide and spread the drop of blood to approximately 4 times its original surface. The slides can then be stained using staining solution after extensive drying. Staining is a colourisation process of blood cells in blood samples that is used for microscopy visual detection. There are several staining methods available for malaria blood smear staining, such as Giemsa stain, Leishman stain and Wright stain. However, Giemsa stain is the widely used staining method for malaria blood smears due to its stability in tropical conditions. Giemsa staining solution stains up nucleic acid, thus red blood cells (RBCs), white blood cells (WBCs), platelets and *Plasmodium sp.* parasites will be colourised differently throughout the process and thus aid in identification of blood cells and the presence of *Plasmodium sp.* parasites. The malaria parasites in a well-stained thick smear show deep red chromatin and pale blue cytoplasm while schizonts and gametocytes are also easily recognizable if present In thin blood smear, the size of the infected red blood cells and the presence of characteristic dots such as Schuffner's dots will be observed (Cuomo, 2009).

Laboratory diagnosis by microscopic examination of stained blood smears is a skill-based manual diagnostic procedure which requires well-trained and competent microscopist to execute the task. The whole process is to determine parasitemia and distiguish between parasitic cells and non-parasitic stained cells such as erythrocytes, white blood cells , platelets and artifacts. The identification of different species in thin blood smears based on the morphological characteristics such as erythrocyte size, shape, crenation, characteristic dots, pigment structure and color at different life cycle

stage is also necessary . A well-trained microscopist should be able to detect the plasmodium species correctly in thick blood smears which is relatively low in parasite density. However, misidentification or error in estimation of the species in microscopic image can still be quite frequent, even in routine microscopy (Breman, Alilio, & White, 2007). This may due to degradation of quality of blood smears with time , poor staining of blood smears, lack of experience in observing the parasitic cell (especially in non endemic area) and other human errors (World Health Organization, 2009) . The accuracy of microscopic inspection is directly affected due to these errors.

According to (Ashraf et al.,2012), accuracy is important in microscopic examination as false negatives can result in untreated malaria patients while false positive may resulted in treating a patient with the wrong treatment or overlook the real cause of the patient's symptoms due to misdiagnosis. Both conditions may caused fatality.

Thus, in order to overcome the issue, many current researches are putting an effort to develop automated malaria microscopic image analysis system by using different image processing algorithm.

1.2 PROBLEM STATEMENT

Visual examination of microscopic images is the most widely used method in studying or analyzing the illness related to infection or viruses. However, it is a time consuming and repetitive task. Microscopic blood smears are prepared one by one for each blood samples. Thus, if there are a lot of blood samples, there will be a lot of microscopic blood smears. The blood smear slides will be kept away after each analysis. However, if the work of the microscopists is being interrupted, the microscopist will need to start all over again from the scratch.

Although visual examination technique using microscopic blood smears have been existed for decades, but there is still a limitation in how well human understand and know about our own body. There is always an emergence of new dicoveries and evolution of virus and parasites. Thus, even though the microscopists are well trained

to execute the job, but they may failed to make an accurate decision when there is a rare case , especially when it happens in non endemic area with less reference of previous cases.

Besides that, since it is a manual visual inspection, thus the result of the diagnosis may be degraded due to limitation of human abilities such as fatigue and inexperience. Different cells in microscopic blood smears are being identified by observing the spatial and intensity information on the blood smears using microscope. Inexperienced microscopists may confuse platelets with malaria parasites (World Health Organization, 2010). Thus, it is important to develop a method that helps in processing a large amount of microscopic blood images in a standardized way to reduce the misdiagnosis.

Automated malaria diagnostic system is an approach to address the issues faced by manual microscopic examination. It is because computer can overcome problems of human error and it decreases the time taken to analyse a blood sample. The foundation of the automated malaria diagnostic system is based on image and signal processing techniques and algorithm as well as mathematical theories. Hence, there is a continuing effort of research on improving and creating a better image processing algorithm for the automated malaria diagnostic system. Many current research is heading towards finding a better algorithm to determine the species of infecting parasites apart from diagnosing the positive or negative status of malaria. The effort is being done in order to match the reality of laboratory diagnosis by microscopy examination to determine the presence of parasitic cell and identifying the species of parasites presented in the blood samples.

Generally, the algorithm of image recognition includes processes such as image acquisition, image enhancement, image filtering, feature extraction and image classification. Hence, exploration can be done on each process in order to improve the automated recognition system. This research is studying and employing a new image analysis algorithm focusing on image filtering and clustering process as an approach for creating a better image analysis system.

1.3 RESEARCH OBJECTIVE

This study provides an overview of application of image filtering and pre-processing techniques in automated malaria diagnosis system and intends to fill a gap in this area by doing so.

The objective of this research is to :-

1) Review on microscopic image analysis techniques for malaria detection.
2) Describe a new approach of enhancing image for analysis by averaging the results of two filters.
3) Evaluate performance of new approach with K-Means clustering, Expectation Maximization and Otsu's threshold algorithm.

1.4 SCOPE OF THE RESEARCH

This research is focusing on the analysis of images of malaria blood samples. The blood samples were presented as the blood smear images, which were originally obtained by using photomicroscopes. Photomicroscope can be formed in its simplest way by attaching an ordinary 35mm camera to the ocular tube of a microscope and record the image of blood smears on photographic smear (House Research Institute). However, the quality of the image generated is highly dependent on the quality of the image produced by the light microscope and the illumination that was being set for the microscope.

The images of that were used for this research are Giemsa stained blood smears images selected from DPDx Parasite Image Library (Center for Disease Control & Prevention). The images obtained from the image library comprised of images of thin and thick malaria infected blood samples of different plasmodium parasite species at different development stage. The collected images are available as JPG format with the size of 300 x 300. Meanwhile, there are some observable differences and characteristics from the images. The overall features and appearance of the cells or objects within the image of blood samples vary from each other. Firstly, there are images that exhibit different development stage of the parasites. Thus, the shape and

size of the parasites are different. Besides that, there are overlapping of red blood cells in some of the images, which form clusters of cells and may be difficult for identification.

Next, the images also shown differences in color tone, contrast and illumination. The images are presented in color tones such as light pink, purplish, blue and yellowish to grey (refer Figure 1.1). Besides that, some of the images show clear contrast between the infected and non-infected cell and some shows high contrast between cells and the cytoplasm (background). But there are also images exhibit low contrast between the cells and cytoplasm.

The images also vary in terms of magnification and resolution. The details of the blood samples were shown differently among the images. Some images showing blood cells in larger size but lack in clarity, some shows clear and good quality image.

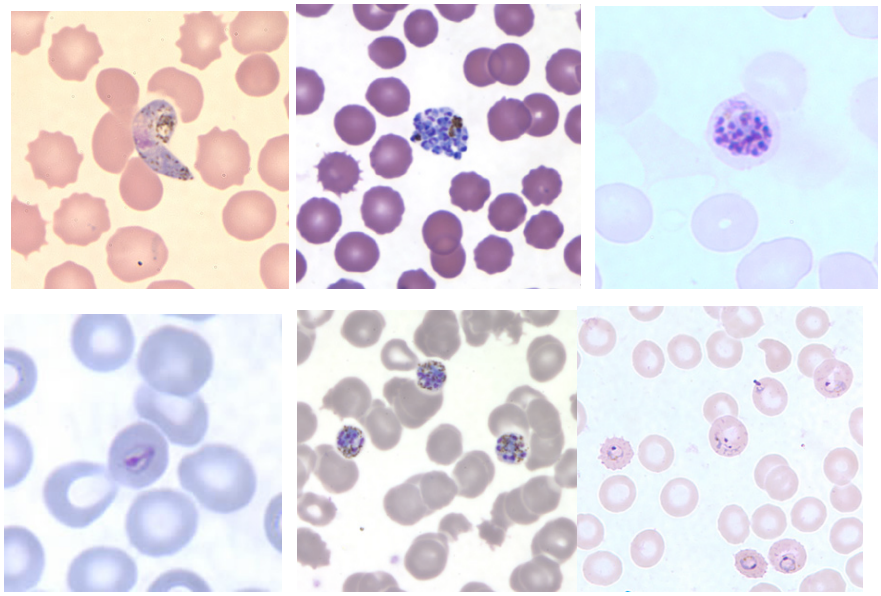


Figure 1.1 Images of blood smears which shown differences (*Image courtesy of CDC's DPDx Parasite Image Library)*

The algorithm of this research is developed in MATLAB. MATLAB is the abbreviation for MATrix LABoratory. It is developed by Mathworks, Inc. to facilitate matrix computations, numerical analysis and graphics viewing. By using MATLAB,

microscopic blood images can be stored within the folder in MATLAB. Besides that, the Image Processing Toolbox can provide algorithms and graphical tools for image analysis tasks including edge detection, image segmentation, image transformation, measuring image features, and etc.

1.5 THESIS ORGANIZATION

Chapter 2: This chapter deals with the literature survey. It brings to the surface the fundamental and important methods that are being frequently employed by the researchers all around the world. Along with it this chapter also highlights and compares the strength and weaknesses of various prevalent techniques. In the light of this comparison this chapter concludes with a brief summary of the proposed method.

Chapter 3: This chapter describes the proposed method for this project. The method that will be discussed in this chapter are image filtering and image clustering techniques. The project development model is also being explained in this chapter. It gives a clearer idea on how will these methods being implemented in this project.

Chapter 4: This chapter is written about how the methodology is being applied on the microscopic blood images to produce the desired results. There are tables and figures that are being included to better illustrate the idea of implementing the method. This chapter also discusses the output obtained throughout the process. It justify whether the desired result is being obtained. Interpretation of the result obtained and future works is also being suggested to enhance the system.

Chapter 5: This chapter concludes the whole project and relates the project with the current situation with the world.

CHAPTER 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

This chapter discusses the topic on image analysis techniques for malaria detection. Other than detection system for malaria, there is also a wide range of previous research which works on automated system for analysis of other microscopic image samples. Thus , in addition, this chapter will also discuss on the application of computer intelligence technique in cell recognition and analysis system. As a conclusion, in this chapter, the techniques will be discussed and compared on its prevalent strengths and weaknesses.

Kumar , Choudhary, Tembhare & Pote demonstrated a framework for an image classification system to positively identify malaria parasites resent in thin blood smears and perform parasitaemia evaluation. An automatic technique was proposed to detect malaria parasite by extracting red blood cell and classify as infected or normal. RGB color space model was employed in this literature by performing the processing on G or B layer. Otsu's thresholding on blue channel was also being adopted to automatically perform histogram shape-based image thresholding and acquire a binary image. Morphological processing was applied in this research after the thresholding process. However, the image used in the research doesn't contain the relevant data and thus it is difficult to evaluate the accuracy of the approach proposed (Kumar, Choudhary, Tembhare, & Pote, 2012).

In a research done by Panchbai and his colleagues, similar to the previous research ,the scheme is based on the used of RGB color space, G layer processing, and segmentation of red blood cells RBC) as well as cell parasites by auto-thresholding with offset value and morphological processing (Panchbhai, Damahe, Nagpure, &

Chopkar, 2012). The thresholding technique that was being employed for this research is Otsu's thresholding method . Different thresholding value were selected to segment the image. Some additional offset values were also selected based on trial and error method for segmenting the images into two categories to complement the insufficiency of using auto-thresholding only. As claimed by the author, the proposed scheme reduced the time taken for malaria detection and the possibility risk of human error. However the result of RBC count was not desirable due to improper illumination during capturing and the variability in microscopic images of blood samples pose significant challenges for the accuracy.

In a research done by Suradkar, he employed image segmentation smoothing processing technique and gradient edge detection technique to detect malaria parasites in image. The determination of positive and negative status of the infected blood is achieved. However, instead of identifying the infecting parasite species and development stage of the parasite, the function of an automated diagnosis system is limited to identify the positive and negative status of infected blood cells only.

Halim and his teammates proposed a technique for parasitaemia estimation in blood smear images by extracting healthy and parasite infected red blood cell. (Halim, Bretschneider, Li, Preiser, & Kuss, 2006). The determination of red blood cells was done based on pattern matching with parameter optimisation and cross-validation against the expected biological characteristics. In a final stage ,the parasitaemia measure was carried out by partitioning the infected and uninfected cells using an unsupervised training-based method. In order to oercome inhomogeneous backside illumination, compensation of imaging variability was carried out successfully beforehand.

Nasir, Mashor and Mohamed proposed an unsupervised color image segmentation of malaria parasites using moving k-means (MKM) clustering algorithm (Nasir, Mashor, & Mohamed, 2012). Instead of processing the algorithm on RGB color space, in this research, the clustering algorithm was implemented on the saturation and intensity objects of HSI color space ,where, H is hue, S is saturation and I is intensity for segmenting. Median filter and seeded region growing area extraction

algorithm was applied for smoothing the image and removing unwanted regions from the image. However, there are still some unwanted regions such as artefacts are still present on the image due to its size after applying median filter. Thus, in the study, the clustered malaria image was proceeded with applying SRGAE algorithm for obtaining the segmented region's size. Finally, only parasite region was reserved in the image and the holes inside the infected cell were filled by employing morphological reconstruction based region filling. The resultant image shows the fully segmented infected cell region by applying MKM clustering on I object. However, some part of the parasite region was eliminated during the process of applying MKM clustering on I object image.

Somasekar, Eswara Reddy, Keshava Reddy and Lai proposed an interactive procedure for malaria detection in their paper. A general framework was described in the paper. Median filter was chosen as the filtering technique in the study for noise removal and smoothing. After that, the cell infected with malaria was extracted by using granulometry. Then , morphological operator was applied to remove the holes inside the blood cells that was resulted frm the extraction. Lastly, the proposed approach in this paper was evaluated and compared with five other existing techniques and framework(Somasekar, Eswara Reddy, Keshava Reddy, & Lai, 2011).

Computer intelligence technique have been evolving and being implemented in biomedical imaging discipline. It serves the purpose to create an automated recognition system. There are several approaches that is being implemented in biomedical image processing such as artificial neural network, K-nearest neighbor classifier, fuzzified neural network and Support Vector Machine. Due to the development of technology, the previous computer intelligence techniques is also being revised and improved to address the disadvantages of previous technique, such as the modified artificial neural network called dynamic artificial neural network (DAN2).

As discussed by M.,Egmont–Petersen, D.,de Ridder and H.,Handels (2002) in their research , ANNs can be the solution to address the need for an adaptive approach or a fast , parallel approach for development of a system . It is because essentially, the

flexibility of ANNs is due to its parallelism in its process. The calculation of ANN is divided among the nodes and each of the nodes send signals and have interaction with each other. Besides that, for network training and testing, ANNs need a database for the purpose to let the network to learn adaptively like human brain. But, since it is being operated in a machine, the training set and testing set must be different for a system to preserve or train its accuracy in image detection. ANNs is simple to be implemented because there are less mathematical calculation compared to other computer intelligence approach because the operation in ANNs are being executed iteratively among the nodes.

ANN also have the ability to capture and represent complex input and output relationships among data. It is because ANN have the ability to be trained to learn adaptively. Furthermore, the network's overall output error can be minimized by adjusting the neuron connection weights and bias value iteratively and it is effective at approximating complex linear functions or the shape of the partition between classes. By adjusting the weight of neuron connections, the mean square error (MSE) can also be reduced and directly reduced the error in the detection. Lastly , ANNs is more optimized for identifying certain cell types. It is when it is being trained with a certain set of data, it can only be used for detecting or recognizing the same type of data when it is being trained.

As the technology evolves , there is a more stable technique than ANNs that is being also implemented ,which is Support Vector Machine (SVM) as studied in (M.Hajek, 2005) . SVM is a binary classifier architecture which consist of ta kernel and a parameter. Its aim is to classify two classes of instances by finding the maximum separating hyper plane between the two. Thus, SVM generalized better. Generally, SVM performs better than ANNs and have similar result with data classified by ANN, but its implementation is costly and therefore cannot be implemented in all laboratories.

Besides that, it is also difficult to find an optimal parameter which yield the best result. Furthermore, SVM is more complicated to be implemented than ANNs. It is because if SVM is to be used to solve classification problem with data which is not linearly separable, a relevant parameter which is expected to mapped into a linearly separable feature space need to be chosen.

In terms of parameters , distance-weighted K-nearest neighbour classifier (Knn-d) is the technique which is free of parameters and it is simple to perform as employed by F.Boray Tek (2006). The performance of Knn-d is related to the covariance matrices. When the difference in the covariance matrices increases, the performance of Knn-d is also increases . However , Knn-d technique is less sensitive to outliers and it relies on a distance measure where there is an uneven weighting of attributes due to different attribute value size. Complexity of computational part increases when there is an increase of number of prototypes . Besides that, by comparing to other methods, it shows that Knn-d requires a higher processing time and memory space .

However, M.& C. proposed a dynamic artificial neural network technique (DAN2) in 2010 which outperform traditional ANNs and it produces training and predicting performances which are more accurate. Unlike traditional ANNs, DAN2 does not need to fix the number of hidden layers in prior, but it is sequentially and dynamically generated until achieving an accuracy level in performance. Besides that, unlike K-nearest neighbor method, training data does not require to be stored in DAN2. It also performs faster than k-nearest neighbor because there is no iteration through individual training samples is needed. Furthermore , DAN2 does not require kernel function experimentation and final selection. A penalty parameter is also not required by DAN2 unlike in SVM. But in DAN2, the output is continuous, but discrete results are needed to perform classification, certain modifications needed to be made on the model to perform classification. Besides that, DAN2 also shows its complexity in training. DAN2 is trained by adjusting parameters based on error value ,but it is difficult to do the selection. DAN2 is not practical for smaller classification data sets as DAN2 should be trained using its error rate, if the data set is small, it cannot determine the most accurate error rate. DAN2 is more complex than ANN. In order to address the disadvantages of DAN2, it needs to be developed into a hierarchical version.

There is another technique ,invariant moment-ANFIS (IMANFIS) , which is being implemented in the research of recognition of human parasite egg images(Esin Dogantekin, 2008). It is a developed model of ANN which is hybridized with fuzzy logic. The training is done using ANN and the performance is about 100%. This

method is used for both image pre-processing and classification stage and pre-processing stage .In IMANFIS, the most important part of this technique for correct recognition of human parasite egg images is pre-processing stage. However, this technique is not suitable in the disease detection system that is proposed in this paper because it classify the images of human parasites egg based on the edge but for the proposed research, the technique should be able to detect the differences in the blood cell.

2.2 CONCLUSION

In a review study by Tek and his colleagues, it was explained that in the point of view of computer vision, a complete malaria diagnosis system must have the ability to perform image acquisition, pre-processing, segmentation and classification task including some functions such as microscope slide positioning, and an automated ,fast ,and reliable focus . In order to perform diagnosis on malaria blood samples, the diagnosis system of malaria requires the ability to detect the presence of parasite in a blood sample by differentiating between non-parasitic stained objects (artefacts, white blood cell, and red blood cell) and malarial parasites. To specify the infection, an additional process of species and malaria parasite development stages identification by differentiating species and development stages is also required if the blood sample is diagnosed as positive. However, the majority of existing malaria-related image analysis studies do not fulfill the requirements . An over-simplified solutions was resulted and it does not applicable to diagnosis directly (Tek, Dempster, & Kale, 2009).

As a conclusion for the review of literature of this thesis, most of the image analysis system approach done by previous researchers are mostly based on color space and ths usage of clustering technique and morphological operation. Most of the research were also done to determine the parasitaemia , instead of identifying the species and development stage of the parasite that infecting the blood.

In this thesis, the proposed technique to be used for the image analysis is by using a new approach of average of results of two filters, The proposed research is done on disease detection based on the usage of pixel intensity information of cell

image data input to determine the feasibility of direct classification on the data. Furthermore , this research tends to address the shortcoming of previous studies in identifying the development stage of the parasite in infected cell by applying the new approach. The proposed framework will be implemented on the microscopic image of malaria infected blood samples of different parasite species in different development stage.

The detailed description of the implementation of the proposed methodology in this research will be discussed further in the Chapter 3.

CHAPTER 3

METHODOLOGY AND IMPLEMENTATION

3.0 INTRODUCTION

This chapter will discuss in details about the methodology that was being implemented for this research. The methodology is proposed to address the shortcomings in the algorithm as discussed in previous chapter. The first part of this chapter will be introducing about the methodology of this research while the second part will be discuss on agile development process, which was being used to develop the proposed methodology. Before the implementation of this methodology is started for the development of the project, there are several aspects and preparation need to be considered.

3.1 PROJECT METHOD

The design of the proposed method is represented in flowchart (refer to Figure 3.1) . A brief pseudocode can be explained as below:

1.Read image
2.Enhance image by contrast enhancement
3.Filter image with 2 different filters
  a)Median filter
  b)High-pass filter
4.Average results of 2 filters
5.Cluster result of averaging 2 filters with different clustering method

a)K-means clustering

b)Expectation maximization (EM)

c)Otsu's threshold

6.Calculate average of the value of each clustered result

7.Detect the edges of averaged clustered object

8.End

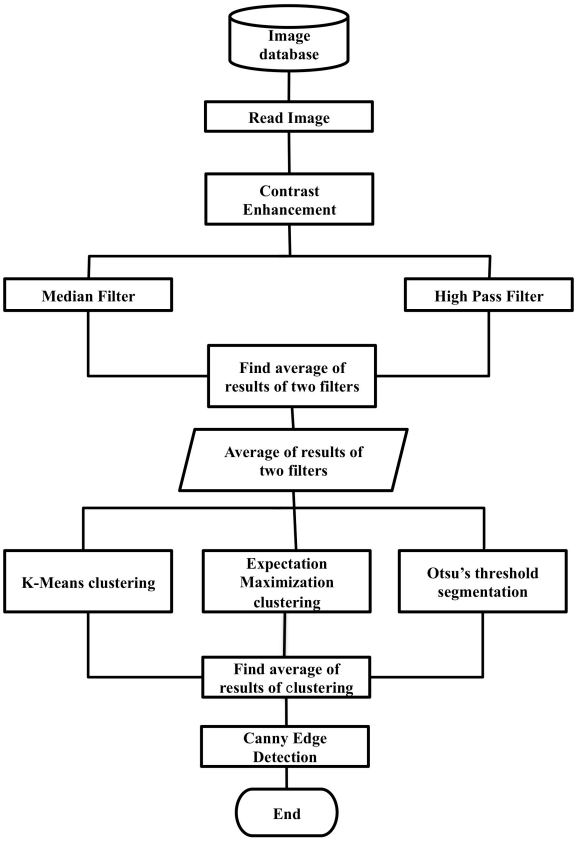


Figure 3.1 Flowchart of proposed algorithm.

In this proposed method, the system starts with retrieving the microscopic image from the dataset. The image was retrieved or read by using the function in Matlab. The command for the operation is 'imread'. The function will be used in association with another function 'imshow' to show the image in the operation. Contrast enhancement was performed on the images to increase the contrast between the background and foreground element. It is because some of the images are in light color tone, thus by using contrast enhancement technique, the clarity of the elements in the image can be shown clearer. The images were transformed into grayscale image by using the green

channel of the image after the enhancement.

In order to achieve the objective of enhancing image for analysis, this research is employing two filters and averaging results of the two filters. Filtering is a procedure to filter some of the image elements and thus sharpen the quality of image by reducing the noises and enhance the image. The filtering techniques that were employed in this research are median filtering and high pass filter. Both filters perform filtering in space domain. The filtering techniques were chosen due to their ability in preserving the edges of the image. A filtering weight mask is used to apply on the image and express its effect on each pixel of the image (Najarian & Splinter, 2006). These type of mask filtering can be expressed as the mathematical expression as :

$$g(x,y) = \sum_{s=-a}^{a} \sum_{t=-b}^{b} w(s,t) f(x+s, y+t)$$

While,
(x,y) = coordinate of pixel ,
g(x,y) = pixel value in the processed image ,
f = original image,
w(s,t) = filter coefficients and the original image

Median filtering is known to reduce the noise without eliminating the edges and other high frequency contents. Like the mean filter, median filter will slide through every pixels in the image by using a filter mask and decide a representative value form each pixel's neighbor values. It will replace the middle pixel value of the values in the filter mask with the median of those values. The median value of each pixel value of the original image was determined by performing the following operations (Najarian & Splinter, 2006) :

Step 1: All pixels in the neighborhood of the pixel in the original image which are identified by the mask are inserted in a list

Step 2 : Sort the list in ascending or descending order

Step 3 : The pixel in the middle of the list (median) is chosen as the pixel value for the processed image.

As defined above, it creates pixel value of the filtered image by sorting on the gray level of the pixels in the mask around the central pixels. However, median filters have certain disadvantages. It gives poor performance when the number of noisy pixels is greater than half of the neighboring pixels because it may be dominated by the noisy elements (Najarian & Splinter, 2006). The median filter was employed in this research by using a 3x3 window for the mask.

The second filtering technique that was applied in this research is high pass filter. The high pass filter also uses a filter mask known as convolution matrix. High pass filter applies negative weighting values as the neighboring pixels and this enhances the regions of high intensity values in the image effectively. Instead of sorting the values and replace the middle pixel value with median value, high pass filter replaces each pixel value by a weighted average of its neighboring pixels. Thus, there is also a possibility to generate negative numbers as the pixel values of the filtered image due to the negative numbers in the applied mask (Najarian & Splinter, 2006). In this research, a 2x2 window was used as the mask for this research. Both of the size of mask used in this research were determined through trial and error method to find the mask best suits the algorithm and images.

High pass filter is one of the sharpening filters which is used to extract the fine details from an image and enhance some blurred details .It is advantageous in extracting edges but not desirable to improve quality of blurred image as it eliminate all important low-pass object which is necessary for improving an image.

After filter the original image with both filter each, the result of the both filtered image was continue by performing averaging of both filtered images. It is because due to the performing qualities of both filters on noisy elements, thus it is being expected that, a better result image can be achieved by doing so. Arithmetic operations were applied on both filtered image to get the image with averaged value pixels of both filtered image.

When the quality of image is being enhanced, it will proceed towards the next step for image segmentation. Image segmentation is an important process for an automated identification system. It aims to divide the image into regions according to the pattern of the image data into its constituent regions or objects. Segmentation of image will be continued until the region of interest is being identified. In malaria microscopic image, segmentation is being performed to enable the clustering of parasite and parasitic cell as well as non-parasitic cell. Three segmentation or clustering method applied in this research are K-means segmentation, expectation maximization and Otsu's threshold clustering.

In K-means clustering, it is assumed that there are K groups of pattern in the data, and the algorithm attempts to find the optimal cluster based on the assumption. Assume that n samples of pattern are given and K groups are to be created from the data using K-means. K-means perform iteratively to find the centers of each cluster and assign each pattern to the cluster with closest center to it. The following steps describe algorithm for K-means:

Step 1 : Randomly initialize the centers
Step 2 : Find the distance of all samples from all centers
Step 3 : Form clusters by assigning each sample to the closest centers
Step 4 : Find the new centers by finding the sample that is closest to the average of all samples in the class
Step 5 : During last iteration, go to step 5 if no sample change its class, otherwise go to step 2
Step 6 : Terminate the process. The final clusters are the output of the algorithm.

The second clustering method that is implemented in this research is expectation maximization (EM). It produces maximum-likelihood in estimating parameters when there is a many-to–one mapping from an underlying distribution to the distribution governing the observation (Moon, 1996). There are two major steps in EM algorithm, which are expectation step and maximization step.

$$E\left[\log b(\mathrm{x})|\, y,\, \theta^{[k]}\right] + \mathrm{c}(\theta)^{\boldsymbol{\tau}}\, \mathrm{t}^{[k+1]} - \log \mathrm{a}(\theta)$$

Where, $t^{[k+1]} = E[t(x)|y, \theta^{[k]}]$, the expectation step and maximization step is :

$$\theta^{[k+1]} = \arg\max c(\theta)^{\tau} t^{[k+1]} - \log a(\theta)$$

In expectation step, the expected value of x data is computed using the estimation of the parameter and the observed data. Then, the data from expectation step is used in maximization step as if it were actually measured data to determine a maximum-likelihood estimate of the parameter. The expectation step may be computed as :

$$x_1^{[k+1]} = E\left[x_1 | y_1, p^{[k]}\right]$$

The EM algorithm may be diagrammed starting form an initial guess of the parameter θ[0] as follows, where every iteration of the EM algorithm increases the likelihood function until a point of maximum is reached :

$$\theta^{[0]} \xrightarrow{\text{E-step}} t^{[1]} \xrightarrow{\text{M-step}} \theta^{[1]} \xrightarrow{\text{E-step}} t^{[2]} \xrightarrow{\text{M-step}} \ldots,$$

The third method implemented for clustering is by means of Otsu's thresholding. The method creates a labeled image from a grayscale image. It selects the optimal threshold by discriminant criterions to maximize the separability if the resultant classes in gray levels into foreground and background object. The procedure is utilizing only the zeroth value from the binary and the first order cumulative moments of the gray-level histogram (Otsu, 1979). The algorithm of this method can be described as the following :

Step 1 : Compute histogram and probabilities of each intensity level

Step 2 : Set up initial class probability and class mean which is computed from the histogram

Step 3 : Iterate through all possible threshold values of intensity

-Update class probability and class mean

-Compute minimization of intra-class variance (same as maximizing inter-class variance)

Step 4 : Desired threshold corresponds to the maximum variance

Step 5 : Compute two corresponding threshold variance

Step 6 : Compute average of the two thresholds

Lastly, there are three threshold images computed from the clustering method. The resultant images were continued with finding an average of the images by employing arithmetic processes. Lastly, Canny edge detection technique was applied to draw the boundary of the segmented object and to differentiate between them. The algorithm of Canny edge detection runs in steps as below:

Step 1 : Smoothing of the image to remove noise

Step 2 : Find the gradients and mark the location where it has large magnitudes

Step 3 : Mark only the local maxima

Step 4 : Determine the potential edges by thresholding.

Step 5 :Determine the final edges by suppressing all edges that are not connected

3.2 AGILE DEVELOPMENT MODEL

There are many system development model available, such as, Waterfall model, spiral model, exploratory model and agile development model. The proposed methodology needed to be developed so that it can be a concrete model for this research. The model of this research was constructed based on agile software development model. In this research, the measure of success of the image analysis system is based on the analysis result of the parasite in the blood sample image. The performance of the algorithm was tested with different clustering method and there is a wide range of possibilities to find the parameter that best suits the algorithm in order to achieve the objective of enhancing the image. Thus, the development model of the system needs to be flexible to enable constantly changing or iterative process. By evaluating the needs of this research, agile development model is the best model to be

implemented to suits the project's development life cycle.

The process of the development model is shown in Figure 3.2 (SERENA, 2007).

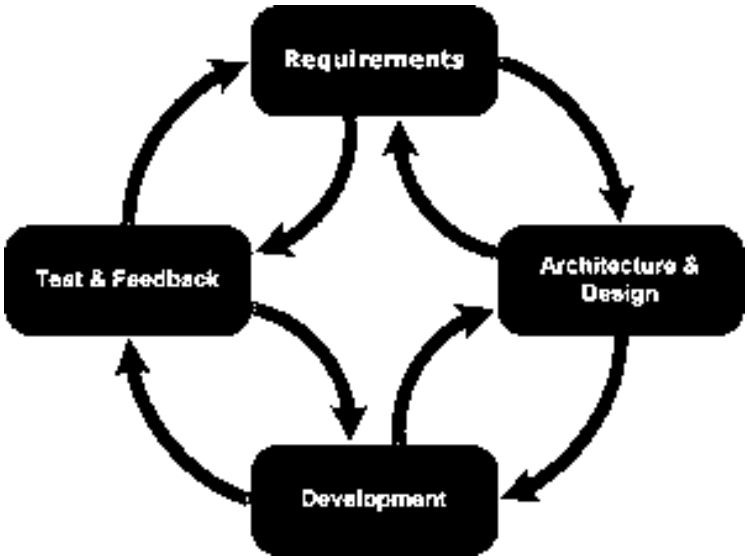


Figure 3.2 Model of agile development process.

Agile software development was initially a philosophy that is put to paper in 2001 with an initial 17 signatories. It was also an important departure from the document-driven software development model, such as waterfall model (SERENA, 2007). Agile software development model is a different approach from a more traditional waterfall model. The progress of waterfall model is made from top to bottom and it emphasizes on the requirement documentation of the system. By comparing to the waterfall model, agile development model deals better with uncertainties, constantly changing requirements and it is less costly and it requires less time.

Based on the model shown in Figure 3.2, the development life cycle of agile model is based on iterative and incremental development. The entire system is not build at once, but it is developed incrementally. The process of the agile system development model consists of development phase like waterfall model such as planning, getting requirements and analysis, design, implementation and testing. The development of system will start with planning of the project and followed by the next steps. But the time taken for each step are short and the steps will iterate for several cycles before the

final product is released. The details of the requirements and functions of the system can be added as the system is being built. Developers can continue on developing the system while testing and analyzing on the system based on the feedback or requirements from the user.

The application of agile development model in this research started with requirement phase as well . During requirement phase, the requirements needed for this research were determined and gathered. Before gathering the needed tools and materials, an initial literature review about the problem domain was done. It is to ensure an initial knowledge and idea about the research was created in order to proceed with the research. Similar previous works and studies were being reviewed to achieve the objective of creating the initial idea. After that, the research proceeded with designing phase. This research is conducted based on the design of the algorithm. Thus, the algorithm of this research was being constructed for the architecture and design phase. The design and architecture of the algorithm was constructed based on the knowledge gathered during initial requirement gathering. The software tools and materials or dataset were determined after reviewing several related literatures and designing the algorithm. Dataset such as numbers and type of images needed and suitable algorithm development platform were decided simultaneously while algorithm for the research was constructed during design phase.

The development of this research's algorithm started after gathering all the needed requirements and tools. In this research, the result of employing the proposed algorithm on the image is the product of the research. The algorithm was tested on the image dataset. Thus, by following the agility of this development model, the development phase and the testing phase can be done iteratively without returning to the first phase. In this research, the performance of the algorithm can be tested and evaluated after each part of the algorithm was done. Meanwhile, during the development of the algorithm was progressing, literature review of the problem domain can still be proceeded at the same time. It enables enhancement on the algorithm in this research from time to time.

3.3 SOFTWARE and HARDWARE TOOLS

This subchapter will summarize the software and hardware that was being used during the development of this research. The implementation of the methodology in developing the project will be discussed further in the next chapter. The image pre-processing and neural network of this research will be constructed by using Matlab software with Image Processing Toolbox. The requirements of the research are summarized in Table 3.1 while Table 3.2 shows the software needed for the research while Table 3.3 shows the hardware tools needed in this research.

Table 3.1 Requirements Needed for Disease Detection Using Microscopic Image

No.	Requirements	Description
1	Microscopic images	The images are the main data to provide the training and testing set of the neural network. It acts as a database for the system in recognize and detect the disease.
2	Matlab with Image Processing Toolbox	Matlab is used to process the image for extracting the data and to construct the neural network. The neural network also needed to be trained and tested using the software.

Table 3.2 Software Being Used

No.	Requirements	Description
1	Platform	Matlab R2009b
2	Database	Matlab R2009b
3	Image Processing	Matlab R2009b with Image Processing Toolbox

Table 3.3 Hardware Being Used

No.	Requirements	Description
1	Personal Computer (PC)	Operating System : Windows 7 Professional RAM : 4GB Processor : Intel ® Dual Core ™
2	Database	Matlab R2009b
3	Image Processing	Matlab R2009b with Image Processing Toolbox

CHAPTER 4

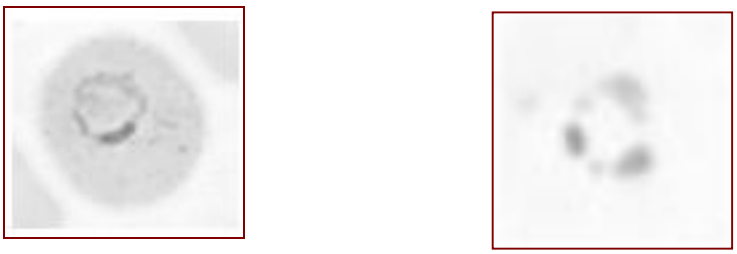
RESULT AND DISCUSSION

4.0 INTRODUCTION

The microscopic images that were selected from DPDx image library was processed by employing the algorithm and techniques that were discussed previously in Chapter 3. In this chapter, the result and findings of the image outputs will be discussed in details. One sample of image each from thin and thick blood smear was chosen as the discussion example in this chapter. Furthermore, the future works on this research will also be suggested to further enhance the algorithm and research.

4.1 OUTPUT IMAGE OF AVERAGING RESULTS OF TWO FILTERS

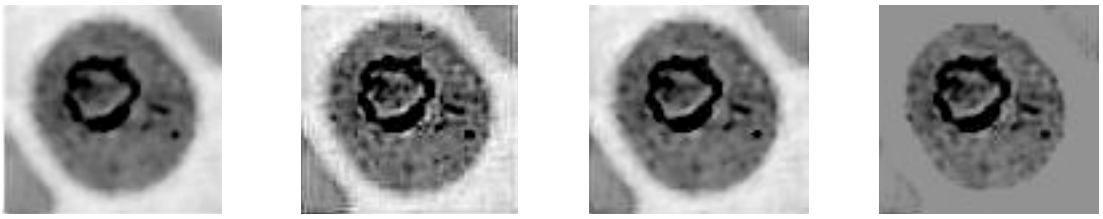
The thin blood smear image chosen for testing with the proposed algorithm is an image of *trophozoite* ring-form of *Plasmodium Ovale* species (refer Figure 4.1(a)) while the thick blood smear image chosen for testing with the proposed algorithm is an image of *trophozoite* ring-form of *Plasmodium falciparum* infected cell (refer Figure 4.1(b)).



(a) **(b)**

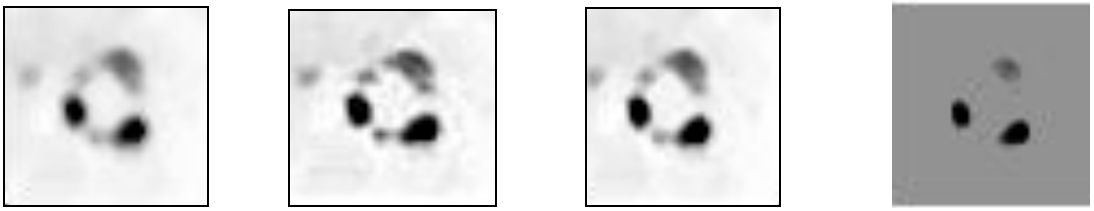
Figure 4.1 Original gray image . ***(Image courtesy of DPDx(CDC)).***

It can be observed that the original gray images are light in color and the contrast between the foreground object and the background object is not obvious. Thus, contrast enhancement was applied on the image to highlight and increase the visibility of the blood cell object in the image. The process was followed by filtration of the image, which median filtering and high pass filtering were employed on each image. The resultant image of the filters can be observed in Figure 4.2 (a) and (b) and Figure 4.3 (a) and (b). Next , the resultant image of the filters were averaged , which was processed as proposed in the new approach.



(a) **(b)** **(c)** **(d)**

Figure 4.2 Image filtered with different filters (thin blood smear). (a) Image filtered with median filter.(b) Image filtered with high pass filter.(c) Image filtered with high pass filter followed by median filter.(d) Averaging the results of two filters. ***(Image courtesy of DPDx(CDC)).***

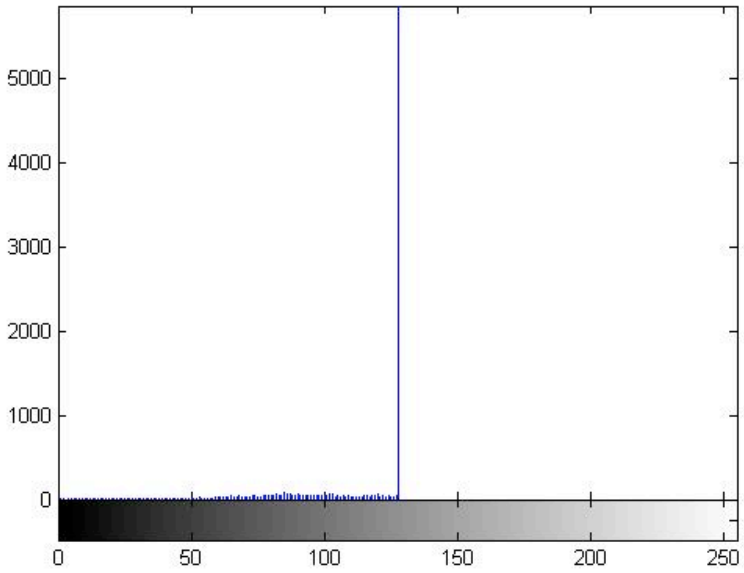


(a) **(b)** **(c)** **(d)**

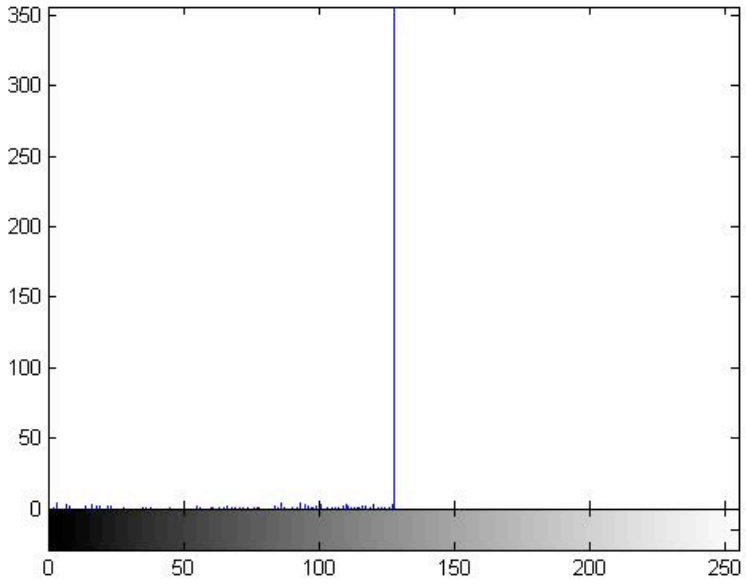
Figure 4.3 Image filtered with different filters (thick blood smear). (a) Image filtered with median filter.(b) Image filtered with high pass filter.(c) Image filtered with high pass filter followed by median filter.(d) Averaging the results of two filters. ***(Image courtesy of DPDx(CDC)).***

From the image produced (refer Figure 4.2 (d) and Figure 4.3 (d)), it can be observed that the resultant image of averaged with two filtered images produced an image that is lower in contrast and brightness. In both figure, it can be observed that the

image becomes darker and the blood cell within the image is not highlighted. In Figure 4.3(d), the noisy elements of the edges are missing. However, the region of the parasite is clearly distinguished from the background even though the whole parasite region was not highlighted.



(a)



(b)

Figure 4.4 Image histogram of averaging results of two filters .

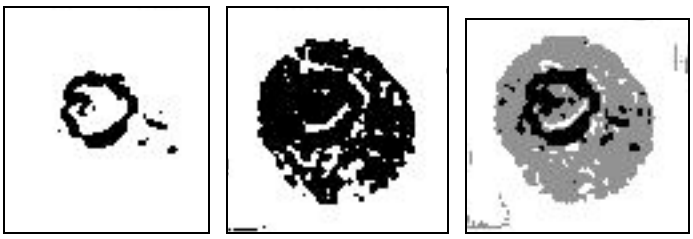
(a) Histogram of average result of Figure 4.2(d).

(b) Histogram of average result of Figure 4.3(d).

Based on Figure 4.4, it can be observed that the intensity of pixel values of the image were divided by two. All of the intensities are moved towards the dark region. That explains the reason for the resulted image becomes dark. However , the intensity distribution of the pixels are remained. There was a certain extent of contrast stretching within the image histogram.

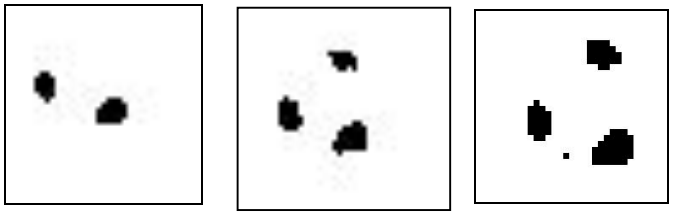
4.2 OUTPUT IMAGE OF AVERAGING RESULTS OF CLUSTERED IMAGE

This section shows the resultant image of clustering the resultant image of averaging image form two filters. The images were clustered by using expectation maximization algorithm , k-means clustering and Otsu's thresholding. By trial and error method, 3 classes was assigned in each clustering technique as the initial number of classes for the clustering , which represent the classes for parasite, red blood cell that was occupied by the parasite and background object.



(a) **(b)** **(c)**

Figure 4.5 Image clustered with different method (thin blood smear).
(a) Image clustered with Expectation Maximization.(b) Image clustered with K-Means.(c) Image clustered with Otsu's method.



(a) **(b)** **(c)**

Figure 4.6 Image clustered with different method (thick blood smear).
(a) Image clustered with Expectation Maximization.(b) Image clustered with K-Means.(c) Image clustered with Otsu's method.

The image clustered with Expectation maximization provides the best result in segmenting the parasite compared to other clustering method (Figure 4.5(a)). However, the resultant image of clustering by using Otsu's threshold method shows the classes of image segmented as defined in the operation, which shows 3 classes of objects. Form the resultant image observed in Figure 4.6, it shows a highly segmented image which shows the parasite in the thick blood smear image. However, these images can be considered as over-segmented as the ring form of the parasite cannot be formed or observed from these images.

The image shown in Figure 4.6 (a) only shows two region of segmented parasite, while it should have been three. Thus, in order to evaluate on the effect of classes initialization, an experiment was applied on expectation maximization by assigning number '10' as the classes of object. The result of the trial was shown in Figure 4.7. It shows three segmented regions of parasite just like the image segmented by using K-Means and Otsu's algorithm.

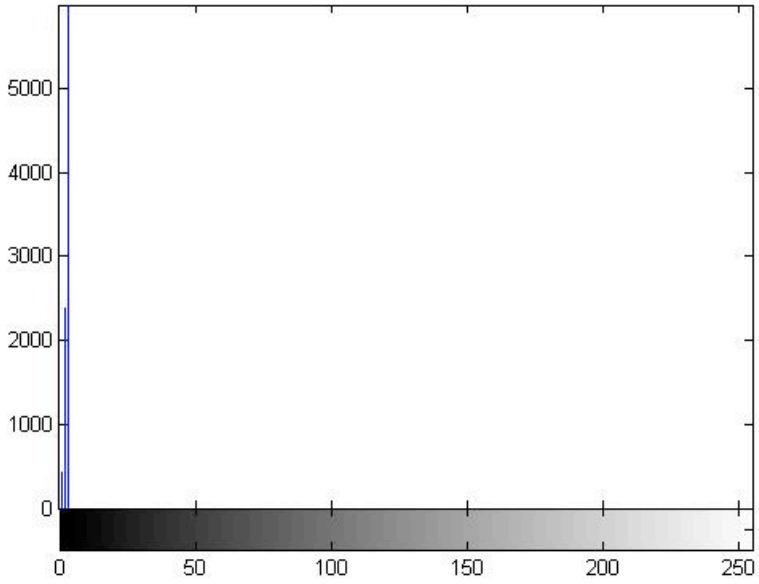
Figure 4.7 Enhanced image of Figure 4.5(a).

After clustering the resultant image of averaging two filters, the images produced by each clustering techniques were again undergoes averaging operation. The image produced by averaging the resultant image from different clustering method was shown in Figure 4.8. Generally, the images were transform into an image with only black color after averaging all the clustered images and no object can be observed from the image. But, when it was being observed carefully at a certain angle while it was displayed on computer screen, there is still an object within the image, but it was too dark to be seen.

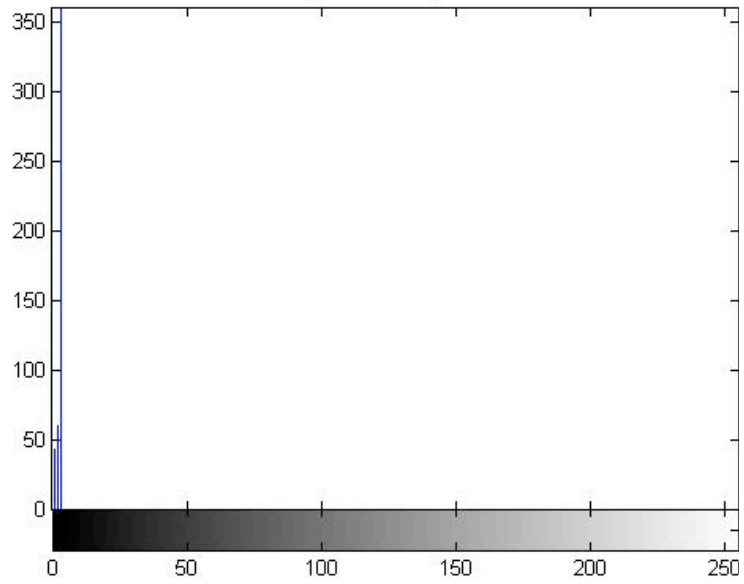
(a) (b)

Figure 4.8 Image of averaging resultant clustered image. (a) Resultant image of averaging clustered images of thin blood smear. (b) Resultant image of averaging clustered images of thick blood smear.

The behavior of the resultant image of averaging the clustered images can be explained by using its histogram. Figure 4.9 shows the respective histogram for resultant images in Figure 4.8. It can be observed that the intensity values of the image shift towards the darker region of lower value again after averaging the clustered image. Thus, the resultant image of averaging the clustered image becomes dark. The images in Figure 4.8 shows a black colored image when observed with naked eyes because the intensities are moved towards a value of nearly zero. However, there are still some other objects presented within the image if evaluated by using the histogram.



(a)



(b)

Figure 4.9 Image histogram of averaging results of three clusters .

(a) Histogram of resultant image of Figure 4.8(a).

(b) Histogram of resultant image of Figure 4.8(b).

4.3 OUTPUT IMAGE OF CANNY EDGE DETECTION

The resultant image from averaging the clustered image using three clustering method was proceeded with canny edge detection in order to visualize the result of averaging clustered images. Figure 4.10 shows the resultant image of canny edge detection for both images of thin and thick blood smears.

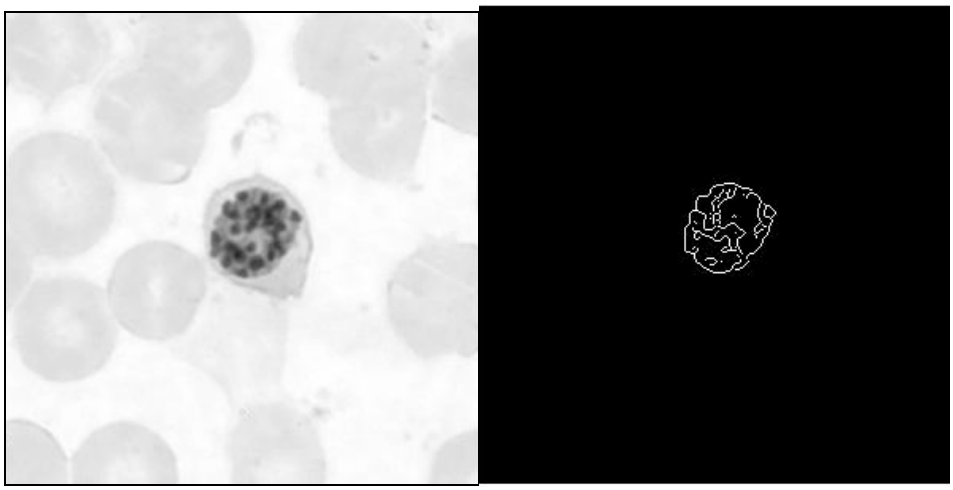
(a) **(b)**

Figure 4.10 Output image of Canny edge detection. (a) Image of thin blood smear. (b) Image of thick blood smear.

By using canny edge detector, it shows that the image of thin blood smear was over-segmented because the objects within the infected cell were not distinguished properly. The region of parasite can be classified and shown on the image, but it was still disturbed by the presence of other objects within the cell. Thus, in order to enhance the result, a trial on modifying the initial classes for clustering was done again and a more desirable image was achieved. The resultant image was shown in Figure 4.11. However, there are still some images that worked well with the algorithm without any modification, for example, the images shown in Figure 4.12. Thus, it can be conclude that this algorithm may be lack of robustness in detecting the differences of the images. The differences of the images were discussed earlier in Chapter 1. Secondly, from the resultant image of canny edge detection for thick blood smear, it can be seen that the three parasite regions were segmented, but it is still lacking because the ring form was not formed in this image with only the three parasite regions. The algorithm needed to be enhanced to enable its applicability in recognizing the development stage of parasite.

Figure 4.11 Enhanced image of Figure 4.10(a).

(a) **(b)**



(c)

Figure 4.12 Output image of Canny edge detection with its original gray image.
(a) Cropped image of thin blood smear of *Plasmodium Ovale* species in trophozoite form.
(b) Cropped image of thin blood smear of *Plasmodium Falciparum* species in older trophozoite form.
(c) Image of thin blood smear of *Plasmodium Falciparum* species in schizont form.
(Image courtesy of DPDx(CDC)).

The focus of this research is to enable the identification of the parasite species and development stages of the parasite by enhancing the image for analysis. However,

the resultant image shows some disadvantages of this algorithm which produces a darken image. The brightness of the image is decreased due to the intensity level are moved towards darker area while remaining certain degree of contrast stretching. The main reason is due to the operation of averaging the results. From the results shown in the images and respective histograms, it can be observed that whenever the average operation was applied, the intensity of the images will shift nearer towards a smaller value which presenting a darker color. It can be explained further with the concept of mathematical operation of averaging or division. By explaining mathematically, it can be understand that division of more than two values causes a smaller resultant value compared to its original value. Thus, the same concept can be applied in this research.

However, most of the images that produced acceptable and desirable result are originally light in color or even in grayscale. It is because high pass filtering and median filtering reserved certain amount of noisy elements in the image while sharpening the edges of the objects in the image. Meanwhile, the contrast enhancement processes that was performed on the image also increases the perceptibility of the objects within the image by enhancing the brightness difference between the foreground objects and backgrounds.

In the context of clustering, the most important variable that manipulating the result is the number of classes defined within the algorithm of clustering technique, whether it is an expected value of classes within the image or an initial declaration of number of classes. The function of clustering method is to categorize the objects within the image into respective classes or groups. Thus, by finding or assigning the most suitable value for the number of classes, the chances of getting the desired result will be higher. In this research, the images used are microscopic images of malaria infected blood samples. Other than malaria parasite or malaria infected cell, there are also other objects or components presented in the blood sample, such as white blood cell, healthy red blood cells, artefacts and the cytoplasm of the cell. It was a challenging task in assigning the most suitable value for the classes, thus it was done by trial and error method to determine the value.

As a conclusion, for the future work, enhancements are needed for the proposed image analysis algorithm in this research. The current algorithm was done on green channel of RGB color space, but in the future, it can be tested on other color space according the literature review that was being done. A further study needed to be done on the effect of combination of average operation and other mathematical or arithmetic operation on an image. Besides that, the focus of the algorithm can be shifted towards finding a more robust algorithm in finding the number of classes for clustering technique.

Furthermore, after the evaluation and comparison of the algorithm in this research based on previous review of literature, it is being found that this algorithm may not be executing the diagnosis or malaria parasite detection task like what it should be in manual microscopic analysis. Thus, further research should be done to improve the whole image analysis system to be completed and achieving malaria diagnosis task without relying on further manual inspection on the analyzed image.

CHAPTER 5

CONCLUSION

5.0 CONCLUSION

Malaria is a parasitic disease that has been affecting millions of people around the world. Due to the effort of eliminating this disease by cooperation of organization such as World Health Organization (WHO) and government, malaria disease was under-controlled in some countries while some are fully eliminated form malaria. However, there are still some countries which categorized under high endemic area , especially in Africa (World Health Organization, 2012). One of the reasons which causes a slow elimination in the high endemic countries is because of insufficient of clinical related diagnosis devices, laboratories and professionals.

Besides that, the procedure of diagnosing malaria disease is also a labor intensive task, especially in laboratory diagnosis by using microscopic visual inspection. It may takes a long time in preparation of blood smear samples and human error may increases the whole diagnosis process and risk. However, it is an important procedure that cannot be ignored in the full diagnosis of malaria infection due to its ability to provide a determination in specifying the species that infected the person.

There are four common species that infected on human, which are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Each species exhibits different morphological characteristics within the infected cell. But , there are certain similarities among each different species. That is one of the reason that increases the challenge to correctly determining the species in laboratory diagnosis. Different parasite species also poses different scale of risk in causing fatality. *P. falciparum* is the deadliest species among

the malaria species that infecting human and causes most of the recorded death cases. Subsequently, the inspection on different development stages of the parasite also plays an important role in malaria diagnosis. It is because the clinician can determine the severity of the malaria infection in a person based on the development stages of the parasite.

Furthermore, each species of parasite needs specific drugs and treatment to deal with. Incorrect prescription of drugs and treatment may not only mistreat the disease, but will also cause death in a person. It is because in the case of true positive, the parasite cannot be eliminate effectively in a person without the right prescription and will continue infecting more cells in the body and causes death, while in the case of false positive, it may cause death because of side effect of the drugs and because of the person is not treated with the right way. Thus, a specific and correct diagnosis is very important in malaria diagnosis and treatment.

Due to the fact that manual microscopic inspection is a difficult task, a computer aided diagnosis system may be the solution for malaria diagnosis and detection. Thus, researches were being conducted to address the issue. There were researches done on determining parasitaemia in micorscopic blood samples , analysis of malaria microscopic blood samples and also creating automated malaria diagnosis and classification system using supervised and unsupervised technique.

This research was conducted for image analysis of detecting malaria in microscopic blood images. The images that were used for the analysis are thin blood smears and thick blood smears images of malaria infected blood samples. It was expected that this research can contribute an effort in solving the issue in image analysis for detecting malaria.

Before the research was started, previous and existing literatures were being studied. Based on the literature reviewed, there were certain extent of shortcomings in current research of malaria diagnosis system. Most of the presented papers described a framework to determine the parasitaemia in blood samples and also determine the positive and negative status of the malaria blood samples, but it was insufficient in

providing function to determine the species and development stages of the parasite.

In order to bring some enhancement in the microscopic image analysis of malaria and to ensure that no manual approach is needed for this image analysis, this research was conducted on a new approach by averaging the resultant image from two filters. There are several objectives that were needed to be achieved to ensure the success of this research. The objective are written as below:

1) Review on microscopic image analysis techniques for malaria detection.
2) Describe a new approach of enhancing image for analysis by averaging the results of two filters.
3) Evaluate performance of new approach with K-Means clustering, Expectation Maximization and Otsu's threshold algorithm.

The first objective of this research is to review on microscopic image analysis techniques for malaria detection. This objective was achieved by studying on existing papers which is conducted on analysing and classifying microscopic image of malaria infected blood samples. Most of the researches reviewed were conducted on analysing image in color space , segmenting image by autothresholding and applying morphological operation. There are some differences among the researches in terms of objective and number of microscopic image used. But, all of the researches aimed to provide an alternative way for malaria disease diagnosis and detection by conducting the studies. However, there are still some improvement needed to be made in order to achieve the objective to enable the proposed framework to replace current manual malaria diagnosis system. It is because most of the researches were done on determining the positive and negative malaria infection status of blood sample, but does not provide the function of identifying species and developement stage of infecting parasite.

Besides that, a review on literature presenting a computer intelligence approach in analysis microscopic images were also being done as it can be another useful sub-element in creating a malaria diagnosis system. There were a lot of works that applying artificial neural network (ANN) in automated image analysis system, but there were also some new approaches presented , such as dynamic artificial neural network (DAN2) and

support vector machine (SVM).

In order to address the shortcomings in malaria image analysis of previous work, this research was conducted to achieve the second objective, which is describing a new approach of enhancing image for analysis by averaging the results of two filters. The implementation of the approach was described in detailed in Chapter 3. The two filters selected for this research are median filter and highpass filter. The filters were being chosen due to their abilities in preserving edges. Meanwhile, median filter can remove unwanted noisy element and smoothing the image as well. The proposed approach was implemented on microscopic images of malaria infected blood samples selected for DPDx image library (Center for Disease Control & Prevention). The resultant image of the new approach is poor in brightness , but preserved contrast to a certain degree. The result of the images were presented in Chapter 4 . Besides that, the framework of the image analysis algorithm in this research also includes other image processing method to enhance the image and complement the whole algorithm, which also addresses the last objective.

The last objective in this research is to evaluate the performance of new approach with K-Means clustering, Expectation Maximization and Otsu's threshold algorithm. The clustering methods mentioned are also described in Chapter 3. The evaluation of the performance of the proposed new approach was being conducted by applying the clustering methods on the resultant image of averaging two filters. The image obtained from each clustering method shown that expectation maximization algorithm produces a clearer but over-segmented image. Besides that, the processing time needed for expectation maximization is also longer compared to other clustering method. In order to further testing the new approach proposed in this research, averaging operation was applied on the resultant image of the three clustering techniques. Lastly , canny edge detection was applied to visualize the average of clustering result and for evaluating the performance of new approach on microscopic image of malaria .

There are some shortcomings in this research where the result of the new approach is not consistent in creating a good clustered image or is not applicable to all the images. Some images get the result of fully segmented parasite infected cell, but

some are oversegmented or undersegmented. In order to enhance the over-segmented image, there was an extra trial and error effort by reducing the threshold value or initial assignment of the number of classes. A better resultant image was produced and the shape of the parasite within the cell can be seen clearer. Besides that , the resultant image of averaging two filters is low in brightness which is due to shifting of pixel intensity towards darker region after averaging. The same shifting also occurred on resultant image of averaging the clustered image. However, the resultant image of fully segmented parasite infected cell is a new discovering and can be proceeded for research in the future. There is also a conclusion can be made from the research that, whenever an average operation was performed on the image, the intensity of the image will be shifted or moved towards the darker region and thus resulting a darker image.

Hence, in the future, the image analysis system can be enhanced by creating a more robust algorithm in screening each microscopic image and determining threshold value for each image and maintaining the brightness of image. Besides that, since there are some successful image analysed by using the new approach, thus, the effect of the new approach can be studied further to find its applicability and condition that enabled a good image result. Furthermore, computer intelligence or heuristic approach can be added to the research to enable an automated and flexible malaria image diagnosis system. The ability of the system on identifying each parasite species and developement stage automatically is required for being usable as an alternative by clinician and microscopist .Thus, more research needed to be done in order to understand the process of manual malaria diagnosis.

As a conclusion, the objectives of this research was achieved , but this research still have a wide space for improvement in the future .

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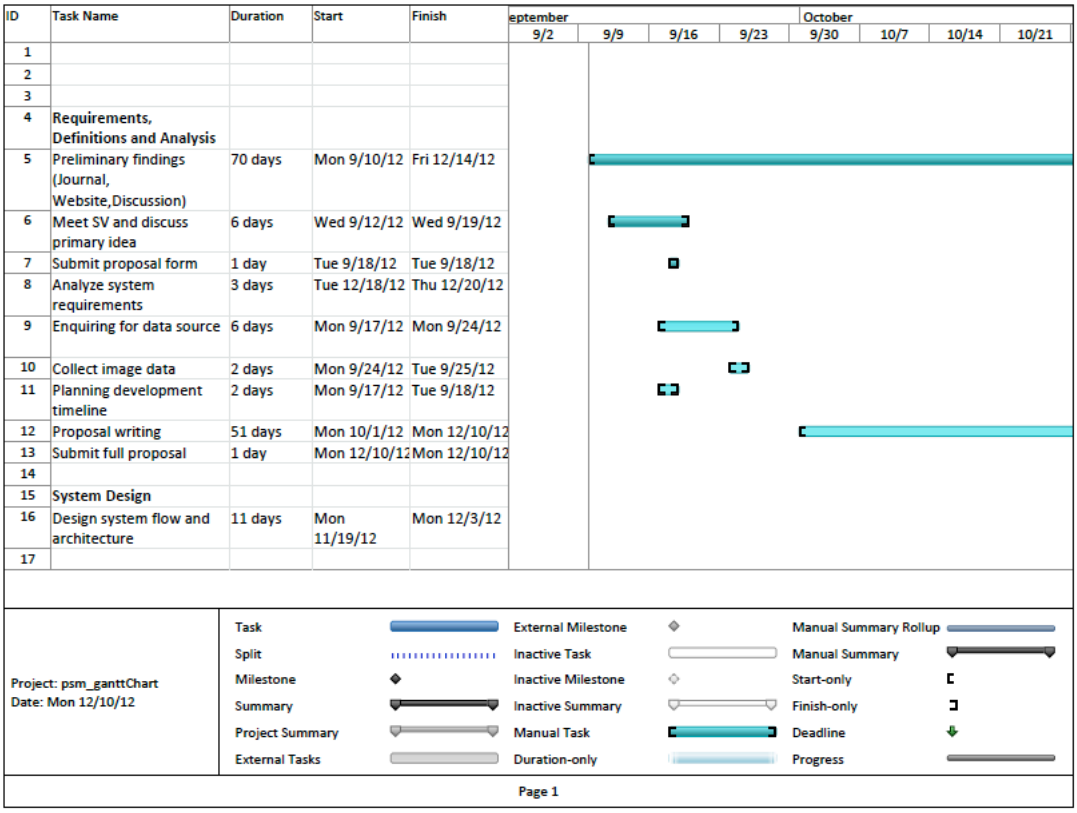
APPENDIX A

GANTT CHART



ID	Task Name	Duration	Start	Finish
1				
2				
3				
4	**Requirements, Definitions and Analysis**			
5	Preliminary findings (Journal, Website,Discussion)	70 days	Mon 9/10/12	Fri 12/14/12
6	Meet SV and discuss primary idea	6 days	Wed 9/12/12	Wed 9/19/12
7	Submit proposal form	1 day	Tue 9/18/12	Tue 9/18/12
8	Analyze system requirements	3 days	Tue 12/18/12	Thu 12/20/12
9	Enquiring for data source	6 days	Mon 9/17/12	Mon 9/24/12
10	Collect image data	2 days	Mon 9/24/12	Tue 9/25/12
11	Planning development timeline	2 days	Mon 9/17/12	Tue 9/18/12
12	Proposal writing	51 days	Mon 10/1/12	Mon 12/10/12
13	Submit full proposal	1 day	Mon 12/10/12	Mon 12/10/12
14				
15	**System Design**			
16	Design system flow and architecture	11 days	Mon 11/19/12	Mon 12/3/12
17				

Project: psm_ganttChart
Date: Mon 12/10/12

Task
Split
Milestone
Summary
Project Summary
External Tasks
External Milestone
Inactive Task
Inactive Milestone
Inactive Summary
Manual Task
Duration-only
Manual Summary Rollup
Manual Summary
Start-only
Finish-only
Deadline
Progress

Page 1

ID	Task Name	Duration	Start	Finish
18	**Implementation and Testing**			
19	Prepare training data	42 days	Fri 2/1/13	Sat 3/30/13
20	Prepare testing data	22 days	Sun 3/31/13	Sat 4/27/13
21	Train neural network	6 days	Sat 3/30/13	Fri 4/5/13
22	Test neural network	6 days	Sat 4/27/13	Fri 5/3/13
23	Analyze and modify	76 days	Sun 1/20/13	Fri 5/3/13
24				
25	**Result and Discussion**			
26	Obtaining result	6 days	Sat 4/27/13	Fri 5/3/13
27	Discussion on project limitation and future enhancement	7 days	Sat 5/4/13	Sat 5/11/13
28	Documentation	7 days	Sat 5/11/13	Sat 5/18/13

eptember | October

9/2 | 9/9 | 9/16 | 9/23 | 9/30 | 10/7 | 10/14 | 10/21

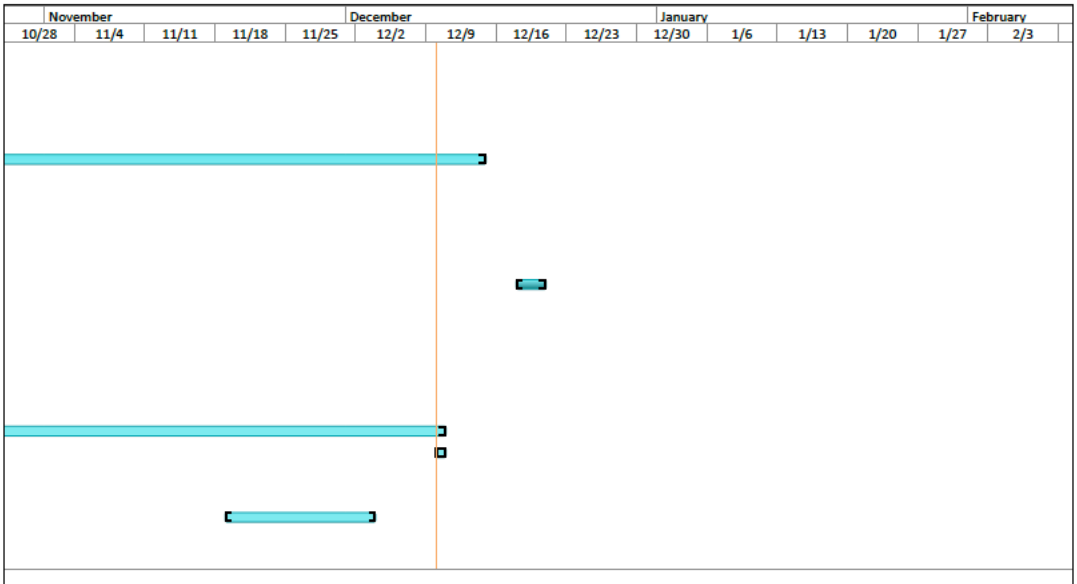
Project: psm_ganttChart
Date: Mon 12/10/12

Task		External Milestone		Manual Summary Rollup	
Split		Inactive Task		Manual Summary	
Milestone		Inactive Milestone		Start-only	
Summary		Inactive Summary		Finish-only	
Project Summary		Manual Task		Deadline	
External Tasks		Duration-only		Progress	

Page 2

November | December | January | February

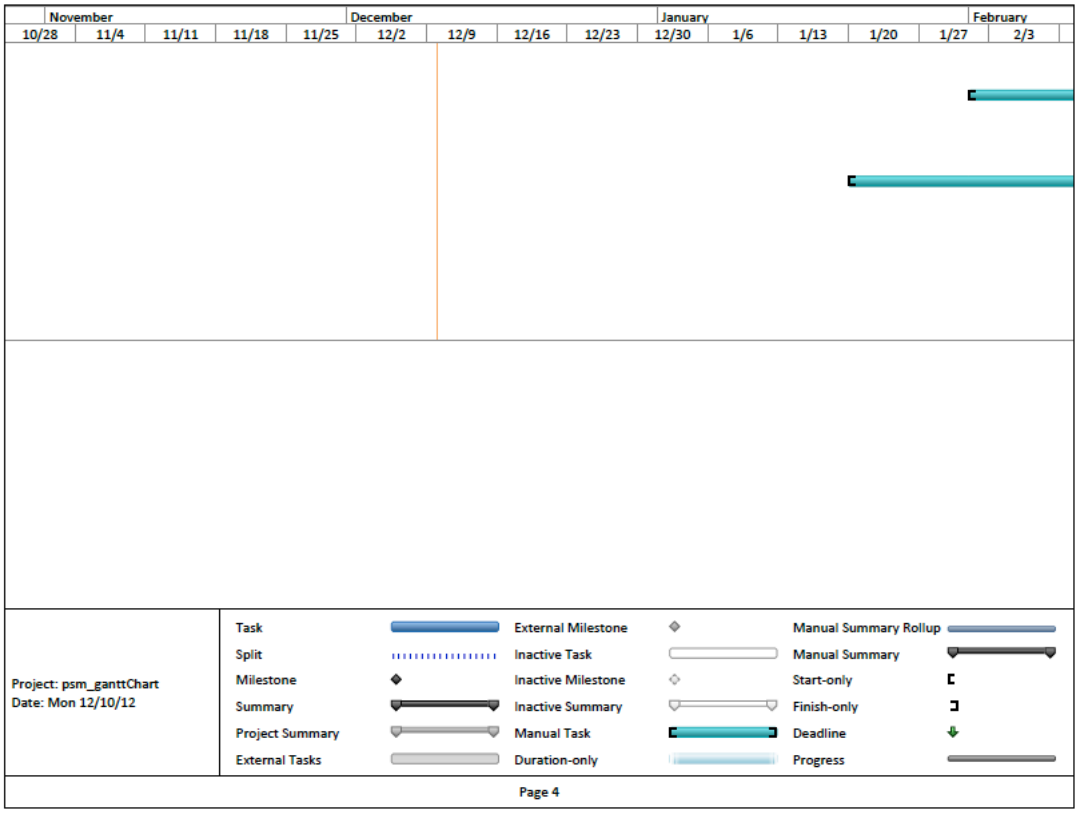
10/28 | 11/4 | 11/11 | 11/18 | 11/25 | 12/2 | 12/9 | 12/16 | 12/23 | 12/30 | 1/6 | 1/13 | 1/20 | 1/27 | 2/3

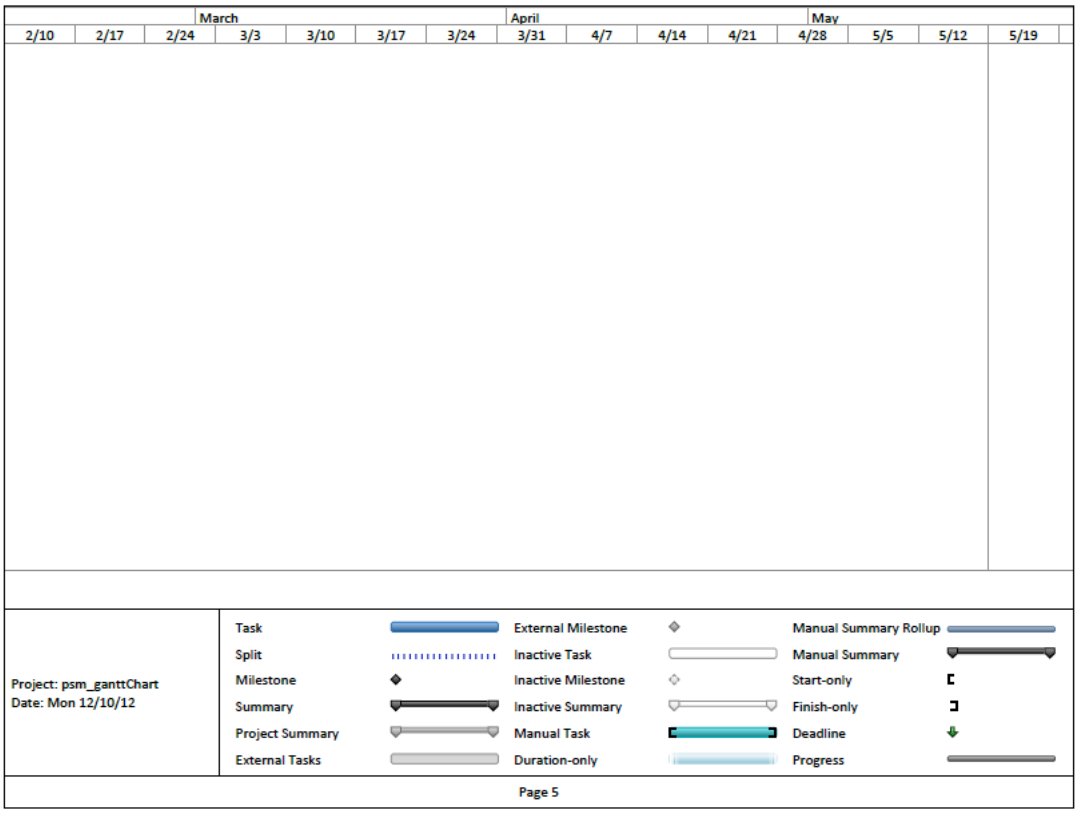


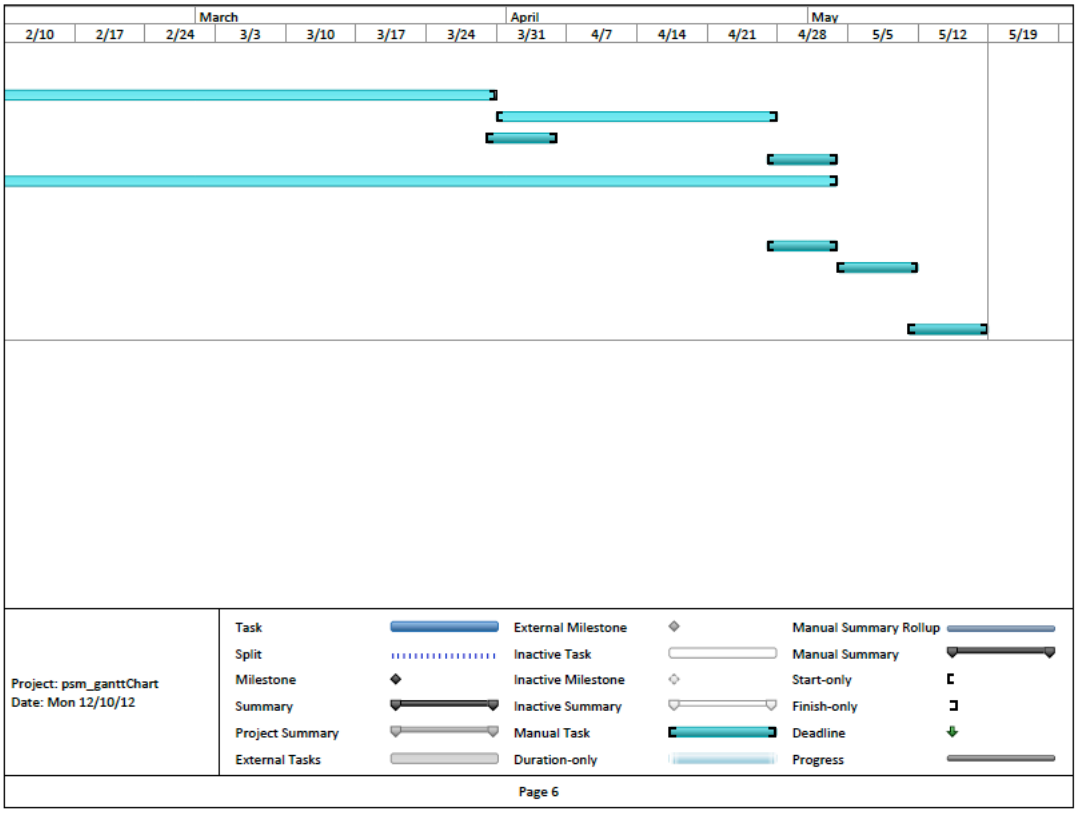
Project: psm_ganttChart
Date: Mon 12/10/12

Task		External Milestone		Manual Summary Rollup	
Split		Inactive Task		Manual Summary	
Milestone		Inactive Milestone		Start-only	
Summary		Inactive Summary		Finish-only	
Project Summary		Manual Task		Deadline	
External Tasks		Duration-only		Progress	

Page 3


November
December
January
February
10/28
11/4
11/11
11/18
11/25
12/2
12/9
12/16
12/23
12/30
1/6
1/13
1/20
1/27
2/3
Project: psm_ganttChart
Date: Mon 12/10/12
Task
Split
Milestone
Summary
Project Summary
External Tasks
External Milestone
Inactive Task
Inactive Milestone
Inactive Summary
Manual Task
Duration-only
Manual Summary Rollup
Manual Summary
Start-only
Finish-only
Deadline
Progress
Page 4


March
April
May
2/10
2/17
2/24
3/3
3/10
3/17
3/24
3/31
4/7
4/14
4/21
4/28
5/5
5/12
5/19
Project: psm_ganttChart
Date: Mon 12/10/12
Task
Split
Milestone
Summary
Project Summary
External Tasks
External Milestone
Inactive Task
Inactive Milestone
Inactive Summary
Manual Task
Duration-only
Manual Summary Rollup
Manual Summary
Start-only
Finish-only
Deadline
Progress
Page 5


March
April
May
2/10
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3/31
4/7
4/14
4/21
4/28
5/5
5/12
5/19
Project: psm_ganttChart
Date: Mon 12/10/12
Task
Split
Milestone
Summary
Project Summary
External Tasks
External Milestone
Inactive Task
Inactive Milestone
Inactive Summary
Manual Task
Duration-only
Manual Summary Rollup
Manual Summary
Start-only
Finish-only
Deadline
Progress
Page 6

APPENDIX B

LIST OF PROJECT FILES

This appendix briefly describes the MATLAB functions , scripts and image files distributed as an integral part of this work. The function files are set in bold . The scripts was created by the author of this thesis, while the functions were created by different authors whom the author would like to acknowledge, for the usage of MATLAB code written . The authors are Damien Garcia (‘Image segmentation using Otsu thresholding’) , Prof. Jose Vicente Manjon Herrera (‘Expectation maximization image segmentation’) and (‘kmeans image segmentation’).

The images were obtained from DPDx Image Library of the Centers for Disease Control (Center for Disease Control & Prevention).

function and script /

EMSeg.m
Computes expectation maximization

kmeans.m
Computes kmeans

otsu.m
Thresholding image with otsu algorithm

psm_algo.m
Script for the whole algorithm for this research

test_EM.m
Script for testing different assignment of number of clusters by applying expectation maximization

test_Kmeans.m
Script for testing different assignment of number of clusters by applying k-means algorithm

test_Otsu.m
Script for testing different assignment of number of clusters by applying otsu’s threshold

images/

M10.jpg,M11.jpg,M12.jpg,M13.jpg,M14.jpg,M15.jpg
Thick blood smear images of *P.falciparum* in ring trophozoites form

M16.jpg,M17.jpg,M18.jpg,M19.jpg,M20.jpg,M21.jpg

Thin blood smear images of *P.falciparum* in ring trophozoites form

M22.jpg,M23.jpg,M24.jpg,M25.jpg,M26.jpg,M27.jpg
Thin blood smear images of *P.falciparum* in ring trophozoites form with Maurer's cleft

M28.jpg
Thick blood smear images of *P.falciparum* in developing older trophozoites form

M29.jpg,M30.jpg,M31.jpg,M32.jpg,M33.jpg
Thin blood smear images of *P.falciparum* in developing older trophozoites form

M34.jpg,M35.jpg,M36.jpg
Thick blood smear images of *P.falciparum* in gametocytes form

M37.jpg,M38.jpg,M39.jpg,M40.jpg,M41.jpg,M42.jpg
Thin blood smear images of *P.falciparum* in gametocytes form

M43.jpg,M44.jpg,M45.jpg
Thin blood smear images of *P.falciparum* in schizonts form

M46.jpg,M47.jpg,M48.jpg,M49.jpg,M50.jpg,M51.jpg
Thin blood smear images of *P.knowlesi* in ring trophozoites form

M52.jpg,M53.jpg
Thin blood smear images of *P.knowlesi* in developing older trophozoites form

M54.jpg,M55.jpg
Thin blood smear images of *P.knowlesi* in gametocytes form

M56.jpg,M57.jpg,M58.jpg,M59.jpg,M60.jpg
Thin blood smear images of *P.knowlesi* in schizonts form

M61.jpg
Thick blood smear images of *P.malariae* in ring form

M62.jpg,M63.jpg,M64.jpg
Thin blood smear images of *P.malariae* in ring form

M65.jpg,M66.jpg

Thick blood smear images of *P.malariae* in trophozoites form

M67.jpg,M68.jpg,M69.jpg,M70.jpg,M71.jpg
Thin blood smear images of *P.malariae* in band form trophozoites

M72.jpg,M73.jpg,M74.jpg
Thin blood smear images of *P.malariae* in basket form trophozoites

M75.jpg,M76.jpg
Thick blood smear images of *P.malariae* in gametocytes form

M77.jpg,M78.jpg,M79.jpg,M80.jpg
Thin blood smear images of *P.malariae* in gametocytes form

M81.jpg,M82.jpg,M83.jpg,M84.jpg
Thick blood smear images of *P.malariae* in schizonts form

M85.jpg,M86.jpg,M87.jpg,M88.jpg,M89.jpg,M90.jpg
Thin blood smear images of *P.malariae* in schizonts form

M91.jpg,M92.jpg
Thick blood smear images of *P.ovale* in trophozoites ring form

M93.jpg,M94.jpg,M95.jpg
Thin blood smear images of *P.ovale* in trophozoites ring form

M96.jpg
Thick blood smear images of *P.ovale* in trophozoites form

M97.jpg,M98.jpg,M99.jpg,M100.jpg,M101.jpg,M102.jpg
Thin blood smear images of *P.ovale* in trophozoites form

M103.jpg
Thick blood smear images of *P.ovale* in gametocytes form

M104.jpg,M105.jpg,M106.jpg,M107.jpg,M108.jpg,M109.jpg
Thin blood smear images of *P.ovale* in gametocytes form

M110.jpg,M111.jpg,M112.jpg
Thick blood smear images of *P.ovale* in schizonts form

M113.jpg,M114.jpg,M115.jpg,M116.jpg
Thin blood smear images of *P.ovale* in schizonts form

M117.jpg,M118.jpg
Thick blood smear images of *P.vivax* in ring trophozoites form

M119.jpg,M120.jpg,M121.jpg,M123.jpg
Thin blood smear images of *P.vivax* in ring trophozoites form

M124.jpg
Thick blood smear images of *P.vivax* in trophozoites form

M125.jpg,M126.jpg,M127.jpg,M128.jpg,M129.jpg,M130.jpg
Thin blood smear images of *P.vivax* in trophozoites form

M131.jpg,M132.jpg
Thick blood smear images of *P.vivax* in ring gametocytes form

M133.jpg,M134.jpg,M135.jpg,M136.jpg,M137.jpg,M138.jpg
Thin blood smear images of *P.vivax* in ring gametocytes form

M139.jpg,M140.jpg,M141.jpg
Thick blood smear images of *P.vivax* in ookinetes form

M142.jpg,M143.jpg
Thin blood smear images of *P.vivax* in ookinetes form

M144.jpg,M145.jpg,M146.jpg,M147.jpg,M148.jpg
Thick blood smear images of *P.vivax* in schizonts form

M149.jpg,M150.jpg,M151.jpg,M152.jpg
Thin blood smear images of *P.vivax* in schizonts form

C_M13a.jpg
Cropped image of M13.jpg

C_M93a.jpg
  Cropped image of M93.jpg

C_M97.jpg
  Cropped image of M97.jpg

C_M32a.jpg
  Cropped image of M32.jpg

APPENDIX C
PLAGIARISM REPORT

This appendix contains screenshots of a general plagiarism report of this thesis. The plagiarism report was generated by using online application called Turnitin (iParadigms, LLC, 1998-2013). It shows the similarity of this thesis with other previous works and other sources.

Turnitin Originality Report

final report by Ann Saw

From Chapter3,4,5,6 or part 2 and 3 (PSM213/2)

Processed on 04-Jun-2013 22:02 MYT
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Similarity Index	Similarity by Source	
31%	Internet Sources:	8%
	Publications:	7%
	Student Papers:	25%

sources:

1 21% match (student papers from 10-Dec-2012)
Class: PSM11213
Assignment:
Paper ID: 291911325

2 1% match (student papers from 16-May-2013)
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http://www.willowfriend.com/All%20My%20Children/AMC%20Pictures/Martin/index_page_01.htm

4 1% match (student papers from 29-Mar-2013)
Submitted to Universiti Malaysia Pahang on 2013-03-29

5 1% match (Internet from 07-Mar-2011)
http://www.malariajournal.com/content/8/1/153

6 < 1% match (publications)
Claudia Kuss, "Estimating Malaria Parasitaemia from Blood Smear Images", 2006 9th International Conference on Control Automation Robotics and Vision, 12/2006

7 < 1% match (publications)
Xing, Xinyu, "A statistical model for fault inference in Wireless Sensor Networks", Proquest, 20111003

8 < 1% match (Internet from 23-May-2012)
http://www.tqm-systems.de/bilder/motivationskarten?b_start:int=60

9 < 1% match (student papers from 30-May-2013)
Submitted to Universiti Malaysia Pahang on 2013-05-30

10 < 1% match (publications)
Yan,. "Region-based geometric active contour for classification using hyperspectral remote sensing images", Proquest, 2012.

11 < 1% match (Internet from 15-Jul-2010)
http://www.scabusa.org/pdfs/forum_99_proc_cbc.pdf

12 < 1% match (publications)
Neuman, . "Image Enhancement", Biomedical Engineering, 2004.

13 < 1% match (publications)
Gloria Díaz, "Infected Cell Identification in Thin Blood Images Based on Color Pixel Classification: Comparison and Analysis", Lecture Notes in Computer Science, 2008

14 < 1% match (publications)
Oladele, Edward Adekola, Louise Ormond, Olusegun Adeyemi, David Patrick, Festus Okoh, Olusola Bukola Oresanya, and Joseph J. Valadez. "Tracking the Quality of Care for Sick Children Using Lot Quality Assurance Sampling: Targeting Improvements of Health Services in Jigawa, Nigeria", PLoS ONE, 2012.

15 < 1% match (publications)
"New Malaria Study Results from S. Ashraf et al Described.", Malaria Weekly (Atlanta, GA), Dec 31 2012 Issue

16 < 1% match (Internet from 24-Mar-2010)
http://eprints.utm.my/2142/1/ShimMongHengMBD2008TTT.pdf

17 < 1% match (Internet from 02-Nov-2012)
http://www.ploscollections.org/article/fetchArticle:?articleURI=info%3Adoi%2F10.1371%2Fjournal.pmed.1000396

18 < 1% match (publications)
Berge, Heidi, Dale Taylor, Sriram Krishnan, and Tania S. Douglas. "Improved red blood cell counting in thin blood smears", 2011 IEEE International Symposium on Biomedical Imaging From Nano to Macro, 2011.

19 < 1% match (publications)
Lori Lentowski, "Chapter 20: Basic Procedures in a Hematology Laboratory.", Hematology in Practice, 20070101

20 < 1% match (publications)

Xinyu Xing, "A Fault Inference Mechanism in Sensor Networks Using Markov Chain", 22nd International Conference on Advanced Information Networking and Applications (aina 2008), 03/2008

21 < 1% match (publications)
Mena, J.B., "State of the art on automatic road extraction for GIS update: a novel classification", Pattern Recognition Letters, 200312

22 < 1% match (publications)
E Awad, "Use of lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, as feed additives to prevent *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum)", Journal of Fish Diseases, 05/2010

23 < 1% match (student papers from 22-Jun-2012)
Submitted to University of Cape Town on 2012-06-22

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