Development of a Colour Normalization and Segmentation System for Malaria Slide Images

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Abstract - **Malaria is a deadly disease where a good portion of the world's population is at risk of getting it. If left untreated the infection will become worse and may even cause death. Thus, it is vital to diagnose the patient who has the parasite as quickly as possible. However, the difficulty in segmenting the infected cell from the non-infected one and poor quality of malaria slide images due to cell staining variations will cause the diagnosis process to be slow. This research paper presents a system that has been developed in order to reduce cell staining variation on the images and the detection of an infected red blood cell. The MATLAB software, R2019b version, will be utilized to develop a GUI system in order to perform colour normalization using histogram specification and an image segmentation process using semantic segmentation which is a deep learning approach. Two semantic segmentation network architectures were chosen for this investigation. The SegNet architecture with its optimum parameters produced the best result for this analysis as the accuracy score is 84.5% and IoU score is 57.3% when a colour normalized dataset is used. In a nutshell, this system can be utilized by an average microbiologist to detect a malaria infected red blood cell more accurately.**

Keywords – malaria slide images, colour normalization, semantic segmentation, MATLAB.

I. INTRODUCTION

As of 2019, 40% of the world's population, which is 3.3 billion people, are at risk of malaria where the majority of the cases occur in Africa. Since the introduction of the Malaria Eradication Program in Malaysia in 1961, Malaysia has battled the spread of malaria. As of 2011, the number of malaria cases has been less than 1 for every 1000 people in Malaysia [1]. The five kinds of malaria parasites that infect humans are *Plasmodium falciparum, P. vivax, P. ovale,* and *P. malariae* and *Plasmodium knowlesi*. The World Health Organization estimated that 409,000 people died of malaria in 2019 and most of them were children [2]. It needs to be noted that an average laboratorian does not have enough experience examining a malaria slide due to the decline in malaria cases in Malaysia, where between the year 2008 to 2017, the number of malaria cases reduced from 6071 to 85 (98.6% reduction) [1]. A laboratorian will face difficulty during examination due to the colour variations that occur in cell staining where he will have difficulty in differentiating an infected cell from a non-infected one during the diagnosis. There is also a problem in the current process of image segmentation, in which a red blood cell that contains malaria parasites are not separated from the ones that are not infected. Thus, a GUI system has to be developed to perform colour normalization on malaria slide images with colour variations and image segmentation on them to separate the infected cells from the non-infected ones using a deep learning approach.

II. LITERATURE REVIEW

A. Color Normalization on Malaria Slide Images

Color normalization is implemented on malaria slide images for staining or illumination correction. Color normalization is implemented in the pre-processing phase [3]. There are a number of transformations and algorithms to obtain color normalized images. The type of algorithm chosen depends on the preferences of the user. The type of color normalization technique that will be used for this research is histogram specification. Histogram specification is a process where, red, green and blue histograms are transformed to match the structure of three specific histograms. This method is more accurate than histogram equalization as it produces more realistic images [4].

Somasekar *et al.* [5], used a modified iterative thresholding to reduce the time consumed for diagnosis. To achieve their objectives, they used a color normalization method on color channels RGB for an accurate enumeration. Before color normalization is implemented color space conversion is carried out to transform a 24-bit RGB image to an 8-bit grey level image. In the year 2021, Aris *et al.* [6] conducted an analysis using the efficacy of *k*-means, fast *k*-means and enhanced *k*-means, algorithms in order to achieve a clean image. The grey world technique was implemented in order to convert the RGB colour model into R, G and B colour components. In the research, the malaria components are easily identifiable in the B component compared to the R and G components.

B. Image Segmentation on Malaria Slide Images

Image segmentation is applied on malaria slide images to highlight the region of interest or more specifically to segment the infected cell from the non-infected one. Deep learning is a subset of machine learning in AI, where the most popular architecture is Convolutional Neural Network (CNN) which helps in evaluating pixel intensity in the original image in which its' value is added on by the convolution matrices known as kernels. CNN could decipher an image without the need to identify special features. Deep learning has been used in the medical arena where extraction of features at the region of interest is done [7]. The type of image segmentation using deep learning that will be utilized in this research is semantic segmentation.

Research conducted by Delgado-Ortet, Molina and Merino et al. [8] in 2020 designed a structure to detect malarial red blood cells. First, erythrocytes are segmented. Then, they are cropped, masked and finally, the infected and non-infected red blood cells are classified. In this research, Semantic Neural Network (SNN) and CNN are created from

scratch using GPU and tested. The SNN was trained and validated using patches where the patches from the original image is matched with the output image. The CNN is trained and validated using the dataset. The SNN had a high accuracy for red blood cell segmentation while the CNN did not show a good generalization power for the training set when it came to classification. Maity and Chatterjee *et al.* [9], implemented an algorithm to identify and classify infected red blood cells. Pixel based segmentation is employed to segment individual red blood cells from the background. CapsNet is used to classify segmented blood cells to identify the species and stages of parasites. The pixel label segmentation starts off by applying multiple filtering settings on a single malaria slide image. ANN will be trained to classify each labelled image. An accuracy of 99.1% was received for the red blood cell segmentation.

III. METHODOLOGY

The main purpose of this study is, to use the method of color normalization and image segmentation on malaria slide images using the deep learning method.

A. Image Acquisition

The malaria slide images were obtained from the Department of Microbiology & Parasitology of Hospital Universiti Sains Malaysia (HUSM). The dataset that was collected contained 117 images of *P. falciparum* species, 37 images of the *P. malariae* species, 29 images of the *P. ovale* species and 40 images of the *P. vivax* species. These images were obtained from the MP-IDB: The Malaria Parasite Image Database for Image Processing and Analysis [10].

B. Image Enhancement

For image enhancement, the *'rgb2lab'* function was utilized to convert the image's RGB color component to L*a*b color space. The adaptive histogram equalization method was used to enhance the image after the conversion. Adaptive histogram equalization is a type of contrast enhancement method which is both automatic and reproducible, and the examiner is encouraged to view only a single image [11]. This enhancement method will help in the colour normalization process as the output image produced will appear more normalized if compared to an image that has not been enhanced.

C. Colour Normalization

For colour normalization, the method that has been proposed is histogram specification. In this method, a malaria slide image was used a reference to carry out histogram specification. Histogram specification is a process in which the histogram of the original image is matched with the histogram of the reference image [12]. To implement this process, one has to separate the colour components of input and reference images. Secondly, one has to extract the image histogram of all three of the colour components of the reference image [13]. Next, one must carry out histogram equalization on the colour components of the input image and of reference image.

The best image that can be used as reference image is the image that that has a white background and contains parasitic or white blood cells as shown in Figure 3.1. The white blood cell, like the parasitic cell, exhibits a purple colour when the Giemsa stain is applied. The presence of the white blood cell

in the reference image causes its colour to be adapted by the parasitic cell in the input image. Meanwhile, the red blood cells that are not infected ought to appear red in colour in order to be differentiated from the infected red blood cells.
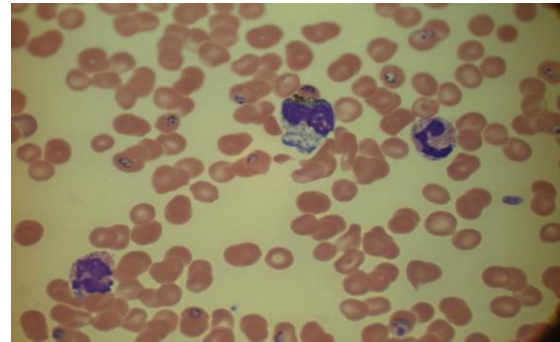

Fig. 3.1 The reference image used for histogram specification [10].

If the use of the colour normalization process is successful, it will greatly aid in image segmentation as the cells could easily be differentiated from the infected to the non-infected one.

D. Image Segmentation using Semantic Segmentation

For image segmentation, two semantic segmentation network architectures will be proposed. The architectures that are proposed uses the deep learning approach. These network architectures are SegNet and DeepLab v3+ Layers. This type of segmentation uses a deep learning formula that associates a label with every pixel in an image. The collected data will be labelled using the Image Labeller app in MATLAB. A datastore will be created where it will contain two categories, one for the original images (Image Datastore) and other for labelled images (Pixel Label Datastore).

When constructing the semantic segmentation network, the Image Datastore and Pixel Label Datastore ought to have two parts, a training and test set. The training set is to train the network to recognize the image objects and test set is to evaluate the accuracy of a network. Later, a pretrained model will be utilized to create an encoder-decoder base which is essential for pixel-level labelling [14].

The first semantic segmentation network architecture that will be utilized is the SegNet Layer using the *segnetLayers* function. The pretrained model that will be used with this network is VGG-16. *segnetLayers* automatically performs the network surgery needed to transfer the weights from VGG-16 and adds the additional layers required for semantic segmentation. The layer graph that is produced contains 91 layers and it is a directed acyclic graph where there is a pattern of up-sampling and down-sampling [15].

The second semantic segmentation network architecture that will be utilized is the DeepLab v3+ layers network using the *deeplabv3plusLayers* function. The pretrained model that will be used with this network is ResNet-50. Like the SegNet architecture, it transfers the weights from ResNet50 and adds additional layers required for semantic segmentation. The layer graph that is produced has 206 layers and it combines an encoder-decoder structure as well as a SPP module for semantic segmentation [16].

E. Evaluating the Trained Network

There are many metrics that can be used to evaluate the trained network by specifying which variables in the dataset, image or class metric tables to compute. For this analysis, accuracy and Intersection of Union (IoU) scores will be the two variables that will be focused on to see the accuracy of the training specifically on how well it segmented the infected red blood cell from the background. The type of metric that will be used to investigate the accuracy and IoU variables is the class metric as the average accuracy and IoU of the infected RBC class for all images can be analyzed.

The amount of correctly identified pixels for each class is indicated by the accuracy variable. Equation 1 shows how to evaluate an accuracy score.

$$Accuracy\ score = TP / (TP + FN) \qquad (1)$$

Intersection of Union (IoU) eliminates false positives by providing statistical accuracy measurements. The higher the value of IoU, the better the training of the network. Equation 2 shows the equation to calculate IoU score. IoU evaluates the similarities between predicted and ground-truth regions for the object of interest.

$$IoU\ score = TP / (TP + FP + FN) \qquad (2)$$

F. Image Post-Processing

After the image segmentation process, post-processing steps are required to fix any faults found within the segmented image. To solve the problem of having minor impurities within a segmented image, the function *'bwareaopen'* will be utilized. Meanwhile, to patch up the holes resulted from segmentation one needs to use the function *'imfill'*. Last, but not least, the *imerode* function is used to erode the segmented image so that unwanted area around the segmented cells can be eliminated.

G. Performing Evaluation

In the performing evaluation stage, a comparison between the segmented malaria slide images that has been color normalized and that has not been color normalized will be made. This is to prove that the color normalized malaria slide images produce better results after segmentation.

IV. RESULTS & DISCUSSION

This chapter demonstrates the results that have been obtained based on the image processing techniques and application that was discussed in Chapter III.

A. Image Enhancement

For image enhancement, only the reference image, that will be used for color normalization, has been enhanced. The malaria slide image that was picked belonged to the *P. falciparum* species. Contrast Limited Adaptive Histogram Equalization (CLAHE) method was implemented to enhance the chosen reference image. Figure 3.1 shows the original malaria slide image and figure 4.1 shows the enhanced version of it.
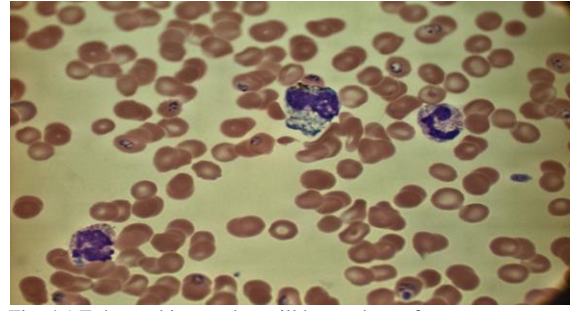

Fig. 4.1 Enhanced image that will be used as reference.

Based on the results obtained in the image enhancement process, CLAHE proved to be a suitable choice in enhancing the contrast of the image. Before the image enhancement process was implemented, the colour of the reference image that was chosen appeared to be faded due to the low contrast issue. This would have affected the colour normalization process where the resulting image produced after histogram specification would not have been more striking and in turn would have negatively affected the image segmentation process.

B. Colour Normalization

For colour normalization, the reference image that was enhanced and described in the previous section will be used to perform histogram specification. The histogram of the other 221 malaria slide images of different species and at various life stages will be mapped onto the histogram of the reference image. Figures 4.2 show the original malaria slide images and its resulting malaria slide image after implementing histogram specification.
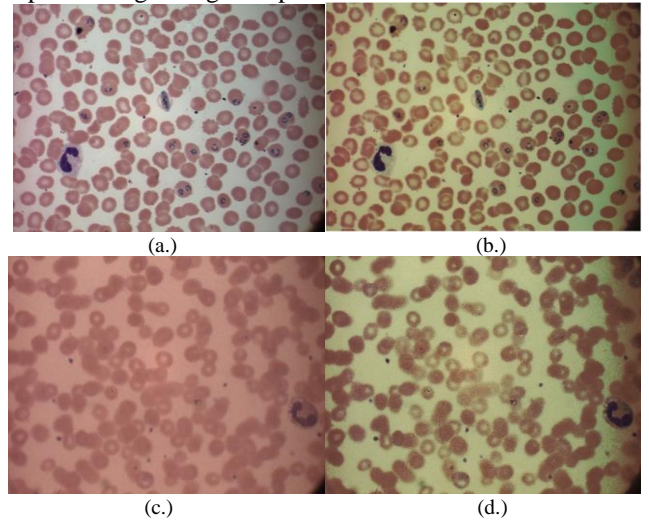

(a.)   (b.)

(c.)   (d.)

Fig. 4.2 (a.) and (c.) show original malaria slide images with different colour variations and (b.) & (d.) show the resulting image after histogram specification was applied.

From the resulting image, it can be concluded that the color normalization process works effectively onto any malaria slide image of different color variations to produce an output, that is more suitable to be implemented in the image segmentation process through deep learning.

C. Image Segmentation

In this section, the process of image segmentation through semantic segmentation using the MATLAB application will be discussed. In image segmentation, the infected red blood cells will be segmented from the rest of

the image. Semantic segmentation is a process in which each pixel of an image is associated with a class label. For this analysis, there are two class labels, the infected red blood cells and background. Then, the stored images are labelled using the image labeler app, which is a pre-installed application found in MATLAB, to two different classes. Figure 4.3 shows the labelled malaria slide image with the pixel label color map. To view the labelled image, the *labeloverlay* function is utilized where the labelled images are overlayed onto their corresponding color normalized images.
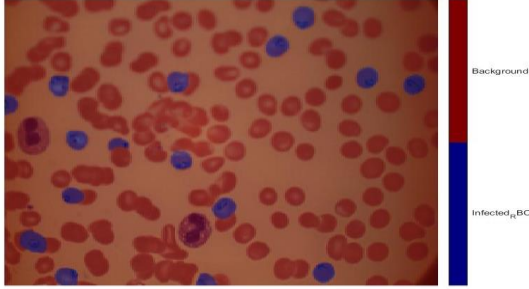

Fig. 4.3 Labelled malaria slide image with a pixel label colour map.

Then, the color normalized and pixel labelled images are resized to a size of 224 by 224. The datasets, both the colour normalized and pixel labelled image dataset, are then divided into two parts. One is used for training and the other for testing.

The *segnetLayers* function is deployed to create a semantic segmentation network based on VGG-16 and the *deeplabv3plusLayers* function is deployed to create a semantic segmentation network based on ResNet50. The last layer of the network graph typically needs to be modified to aid in the transfer learning process. Both the infected red blood cell and background classes have different total number of pixels, where the percentage of distribution are 1.9% and 98.1% respectively. This could contribute negatively to the learning process as the learning will be biased towards the dominant class. Hence, the classes need to be balanced and this could be achieved by using class weighting. Equation 3 and 4 demonstrate on how to obtain class weights. The class weights are then specified using *pixelClassificationLayer* function to give a categorical label for each pixel processed by CNN. This layer will replace the last layer of the layer graph for both the SegNet and DeepLab v3+ architectures.

Image Frequency = tbl.PixelCount / tbl.ImagePixelCount

(3)

Class Weightings = median (Image Frequency) / Image

(4)

The function *trainingOptions* is used to specify the hyper-parameters used for SGDM. The optimum name-value arguments that are chosen for training are different for the SegNet and DeepLab v3+ architectures. The accuracy and IoU scores of four training procedures and its results will be shown in this report. Two of them are, when the training options are exactly the same for both the semantic segmentation architectures and the other two are the optimum parameters for each of the architectures. Data augmentation is carried out to improve the accuracy of the

network by randomly transforming the original data during training. The average time taken for just these four-training progress was three hours.

As discussed in Chapter 3 both the accuracy and IoU variables need to be considered to get the complete evaluation of the segmented RBC. Table I shows the accuracy and IoU scores of the infected red blood cells class label for the SegNet and DeepLab v3+ architecture for both the optimum and controlled parameters. The evaluation is implemented on the testing dataset which has 22 images out of the 221.

TABLE I. ACCURACY & IOU SCORES OF INFECTED RED BLOOD CELLS CLASS LABEL.

	Accuracy of Infected Red Blood Cells Class Label	IoU of Infected Red Blood Cells Class Label
SegNet Architecture with Optimum Parameters	84.5%	57.3%
DeepLab v3+ Architecture with Optimum Parameters	91.5%	52.7%
SegNet Architecture with Controlled Parameters	87%	49.3%
DeepLab v3+ Architecture with Controlled Parameters	93.8%	48.4%

Figure 4.4 shows the black and white image of the ground truth image being compared with the DeepLab v3+ architecture with controlled parameters which has the highest accuracy score and the SegNet architecture with optimum parameters which has the highest IoU score. This comparison is important to show why the accuracy and IoU variables should both be considered.
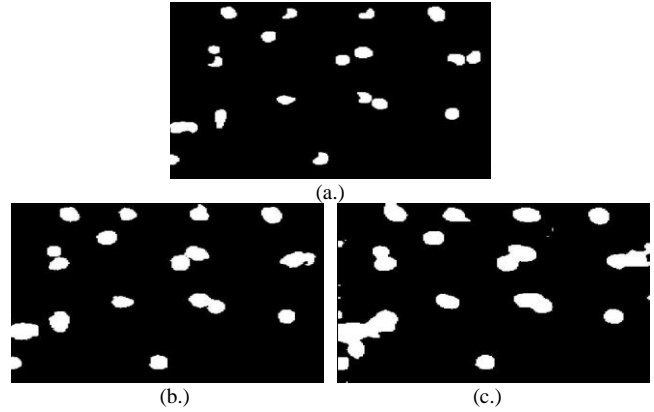

(a.)

(b.)   (c.)

Fig. 4.4 (a.) shows the ground truth image, (b.) shows the predicted image of the SegNet architecture with optimum parameters and (c.) shows the predicted image of the DeepLab v3+ architecture with controlled parameters.

From Figure 4.4, one could observe that the SegNet architecture segmented the infected red blood cells from the background better than the DeepLab v3+ architecture despite having lower accuracy. This comparison was made to show that the IoU variable is essential to evaluate the semantic segmentation and a higher IoU score is more important than a higher accuracy score when it comes to analysing the effectiveness of the network. Therefore, SegNet architecture with optimum parameters performed the best among the four that was investigated in this

analysis. Meanwhile, SegNet architecture with controlled parameters performed slightly better than the DeepLab v3+ architecture with controlled parameters.

After the segmentation process, the images went through post-processing to improve the quality of the segmented malarial cells. As discussed in Chapter III, the functions that were used for post-processing are *imerode*, *imfill* and *bwareaopen*. After the post-processing step, the segmented malaria slide image is masked onto the colour normalized image so that the examiner could identify the infected red blood cells more clearly. Figures 4.5 shows the end result of the segmented malarial cells after post-processing and applying the mask.
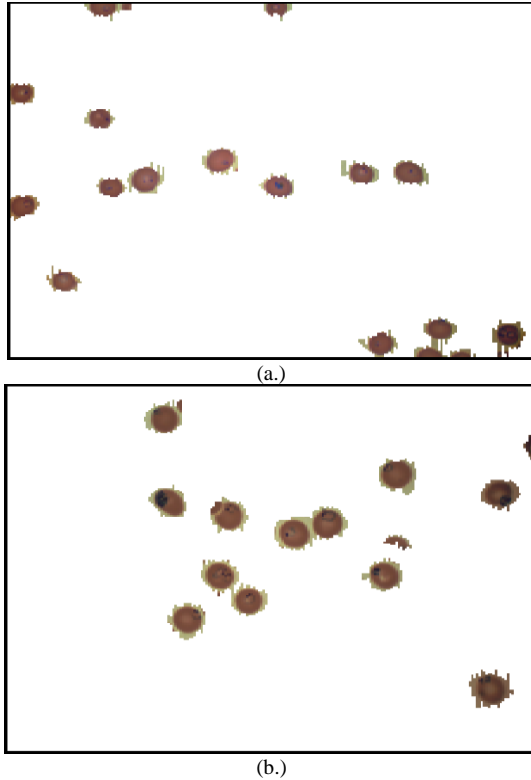

(a.)

(b.)

Figure 4.5 (a.) and (b.) Segmented images of malarial red blood cells after masking.

D. Performing Evaluation

In performing evaluation, the original malaria slide images are compared against the colour normalized ones. The trained network chosen to analyse the accuracy and IoU scores of the original malaria slide images is the SegNet architecture with optimum parameters. Figure 4.6 shows the comparison between the segmented original malaria slide images and colour normalized images with the ground truth image.

Based on Figure 4.6, one could see that the colour normalized images performed better than the original ones. The accuracy and IoU scores that were obtained are 82.3% and 47.3% respectively for the infected red blood cell class label when the original malaria slide images were used instead of the colour normalized ones. Even though, the accuracy score is only slightly lower than the colour normalized images, there is a reduction of 10% for the IoU score which affects the quality of the trained network negatively.
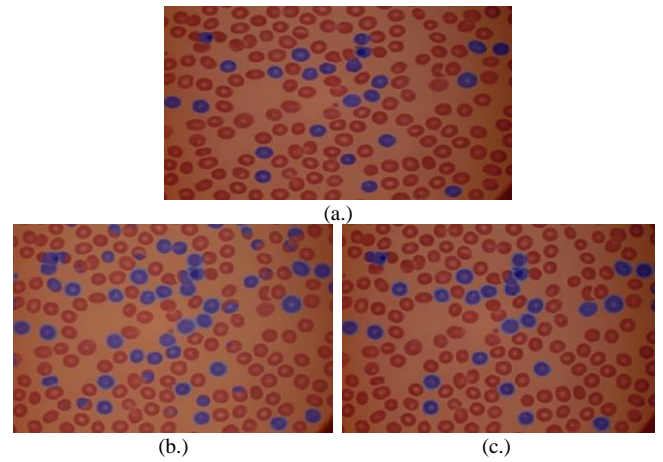

(a.)

(b.)

(c.)

Fig. 4.6 (a.) shows the ground truth image, (b.) shows the segmented original malaria slide image and (c.) shows the segmented colour normalized malaria slide image.

V. Conclusion

Due to the inexperience of an average microbiologist in examining a malaria slide image, an effective system has been introduced to aid the microbiologist in differentiating an infected cell from a non-infected one during diagnosis. A method has been developed to perform a colour normalization procedure on malaria slide images with colour variations and a semantic segmentation procedure has been introduced to separate the infected cell from a non-infected one.

Colour normalization was implemented using histogram specification. As one could observe from Figure 4.2, two different cell staining variations had the same colour after histogram specification was utilized. Four training procedures were conducted to find out the ideal trained network to implement semantic segmentation and this was done by evaluating each trained network using the accuracy and IoU scores. Among the four learning networks, the best was the SegNet architecture with optimum parameters which had an accuracy score of 84.5% and IoU score of 57.1%. The IoU score of this particular trained network was the highest among the four.

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