

## An Intelligent Classification System for Trophozoite Stages in Malaria Species

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**Abstract:** Malaria is categorised as a dangerous disease that can cause fatal in many countries. Therefore, early detection of malaria is essential to get rapid treatment. The malaria detection process is usually carried out with a 100x magnification of thin blood smear using microscope observation. However, the microbiologist required a long time to identify malaria types before applying any proper treatment to the patient. It also has difficulty to differentiate the species in trophozoite stages because of similar characteristics between species. To overcome these problems, a computer-aided diagnosis system is proposed to classify trophozoite stages of *Plasmodium Knowlesi* (PK), *Plasmodium Falciparum* (PF) and *Plasmodium Vivax* (PV) as early species identification. The process begins with image acquisition, image processing and classification. The image processing involved contrast enhancement using histogram equalisation (HE), segmentation procedure using a combination of hue, saturation and value (HSV) color model, Otsu method and range of each red, green and blue (RGB) color selections, and feature extraction. The features consist of the size of infected red blood cell (RBC), brown pigment in the parasite, and texture using Gray Level Co-occurrence Matrix (GLCM) parts. Finally, the classification method using Multilayer Perceptron (MLP) trained by Bayesian Rules (BR) show the highest accuracy of 98.95%, rather than Levenberg Marquardt (LM) and Conjugate Gradient Backpropagation (CGP) training algorithms.

**Keywords:** Malaria parasite; thin blood smears; image processing; classification

### 1 Introduction

Malaria is an acute (sudden) and chronic disease that can threaten people's health worldwide. It has been reported that around 229 million cases and 409,000 deaths worldwide were recorded in 2019 due to malaria, which led to an expenditure of US\$ 3.0 billion to control and prevent malaria at the global scale, particularly within the African Region, South-East Asia, Eastern Mediterranean, America and Western Pacific [1]. Malaria is caused by *Plasmodium* parasites that spread to people through the bites of female Anopheles



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mosquitoes. Malaria parasites can be categorised into five species which are *Plasmodium Knowlesi* (PK), *Plasmodium Falciparum* (PF), *Plasmodium Vivax* (PV), *Plasmodium Malariae* (PM) and *Plasmodium Ovale* (PO) [2,3].

PF, PK and PV are commonly discovered in Malaysia [4,5]. Around 4,630 cases are occurring in 2018, where 89% are from PK. The early trophozoite of PK is identical to those of PF and PV due to similar characteristics such as double chromatin dots, multiple infected RBC and applique forms [6,7]. Therefore, early-stage identification of malaria parasites is an essential factor in preventing and controlling the spread of malaria.

Clinically, visual inspection becomes quite challenging if a microscopic image contains blur effects, unwanted artefacts and a lot of noise in the image [8]. In addition, human perception is not only inconclusive but also not error-free [9]. Furthermore, microscopic examination of blood film for malarial parasites also consumes a lot of time and energy of the microbiologist in deciding the positivity of the sample [10]. Thus, computer-aided systems based on image processing have been developed to assist the visual inspection problems [11–13].

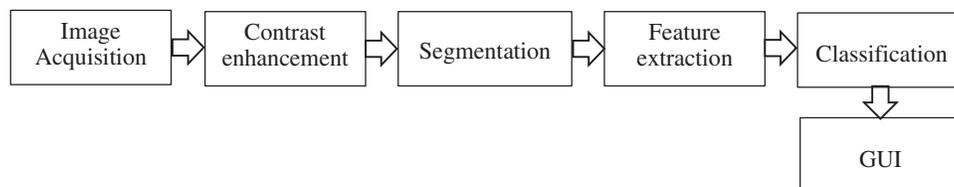
In the studies of image processing and automated classification procedure using a computer-aided system, many limitations have been reported by previous researchers. Most of the classification process only focused on the infected RBCs and normal RBCs classification systems [14,15], classification of PF, PM, PV and PO species with their life cycle stages [16], classification of PF, PM, PV and PO species without identifying the stages in malaria parasite [17], classification of the stage at PF [18–20], PV [21], PM and PO [22], and PF and PV species [23–25], respectively. No previous work has been reported on the automated classification of PK, PF and PV since PK is considered a new and regional species are infecting humans.

Thus, this study proposes an intelligent classification system for PK, PF, and PV Malaria species for trophozoite stages. The system is developed using image processing techniques, including contrast enhancement, proposed segmentation procedure, feature extraction and classification process using MLP-BR to classify the image.

This paper is arranged as follows: In Section 2, the methodology to develop an automated classification system is introduced. Then, Section 3 describes the application of the method and experimental results are presented and analysed. Finally, Section 4 provides the conclusion of this work.

## 2 System Architecture

The system architecture is implemented using five main processes: image acquisition, contrast enhancement, image segmentation, feature extraction, classification, and Graphical User Interface (GUI), as shown in Fig. 1. The proposed approach is introduced and validated using Matlab R2018a with Intel® Core™ i7-7500U CPU @ 2.90 GHz and 8 GB RAM.



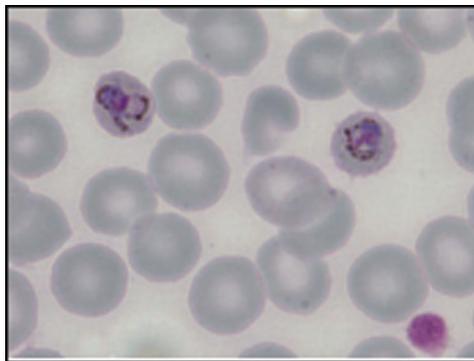
**Figure 1:** The system architecture of the intelligent classification system for malaria parasite

### 2.1 Image Acquisition

Malaria samples were prepared by experts from Hospital Universiti Sains Malaysia, Kelantan, Malaysia. Images were acquired using microscope BX41 connected to Olympus XC50 camera under 100X magnification resolution using immersion oil. The capturing procedure of the sample images has been approved by National Medical Research Register (NMRR), with the register serial number of NMRR-16-1434-31673 (IIR). This study only focuses on differentiating between trophozoite stages of PK, PF, and PV. Moreover, 150 thin blood smear images, including 50 images of each PK, PF and PV, have been captured. Images were saved in the bitmap (BMP) format in  $800 \times 600$  color (RGB) ideas.

### 2.2 Contrast Enhancement

The contrast enhancement method aims to enhance the image's contrast and reduce the color consistency problem of thin blood smear images due to the staining process [26,27]. In this study, the histogram equalisation (HE) method [28] is applied to correct the color intensity of each image by referring to the intensity value of the reference image. Therefore, the microbiologist has validated the reference image as the most suitable image. Fig. 2 shows the reference image using the intensity value for the enhancement process.

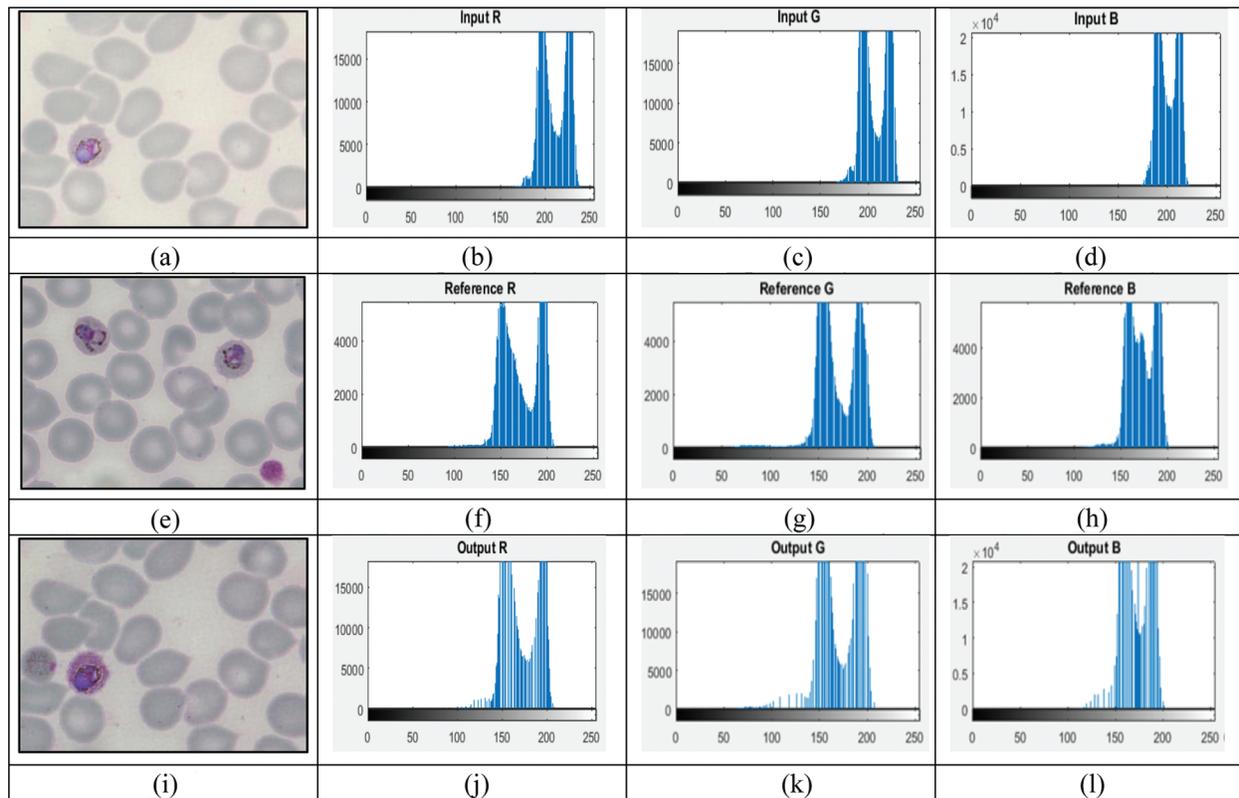


**Figure 2:** Reference image

Using the HE method, the input color image is corrected according to the intensity value of the reference image. The reference image used in the algorithm is fixed and does not change for any color correction process of other photos. Therefore, the reference image contains the best intensity among all samples. Fig. 3 shows the image and histogram, adjusted to the intensity value range as a reference image. Based on Fig. 3, the output image in (i) and histogram in (j), (k) and (l) are similar to the intensity value of the reference image in (e) and histogram in (f), (g) and (h), respectively.

### 2.3 Segmentation

Image segmentation aims to separate the region of interest from the unwanted regions [29]. The proposed segmented procedure has been successfully developed. Eight steps have been implemented in segmentation procedure steps that were successfully done to obtain the region of interest (ROI) by considering the following:



**Figure 3:** The application of the HE method using the intensity value of the reference image. (a) Input image (b) Input R layer (c) Input G layer (d) Input B layer (e) Reference image (f) Reference R layer (g) Reference G layer (h) Reference B layer (i) Output image (j) Output R layer (k) Output G layer (l) Output B layer

1. Load the original color image from Subsection (2.2).
2. Convert the image in Step 1 to a grayscale image.
3. Segment the grayscale image from Step 2 into a binary image using Otsu's method [30]. The foreground is represented as white (assigned to 1), and the background is represented as black (assigned to 0).
4. Apply erosion operation by using structuring element 'disk' to separate the overlapping cells.
5. Segment image in Step 2 using manual thresholding method according to the following equation:

$$I(x, y) = \begin{cases} 1, & \text{if } 50 < I(x, y) < 110 \\ 0, & \text{if } 50 \geq I(x, y) > 110 \end{cases} \quad (1)$$

6. Identify which coordinate in Step 5 overlapped with the cell in Step 4. The rest will be deleted.
7. Infected cells together with an object will be displayed.
8. Apply dilation operation in Step 7 by using the similar structuring element 'disk' in Step 4 to obtain the original size of an infected cell.

Steps 1 to 3 are the segmentation process of the cell images, whereas Steps 4 to 8 are the process for obtaining infected cells.

During the segmentation process, the overlapping image occurred after applying Otsu's method. Therefore, the erosion process has been used to separate the overlapping cells. In Step 5, the manual

thresholding method set 50 to 110 intensity value has been used to find the coordinate location of malaria parasite in the image. This intensity value has been selected based on observation from a microbiologist for 150 images during the experiment.

Fig. 4 shows the resultant images after the proposed segmentation steps starting from grayscale image ((a) to (c)), Otsu's method ((d) to (f)), erosion process ((g) to (i)), finding of coordinate location for malaria parasite ((j) to (l)), and display of infected cell ((m) to (o)).

## 2.4 Feature Extraction

Three features have been extracted, namely the size of infected red blood cell (RBC), brown pigment in the parasite and texture feature using the Gray Level Co-occurrence Matrix (GLCM) technique [31].

### 2.4.1 Size of Infected RBC

The size of infected RBC is based on the sum of white pixel values obtained from the segmented image shown in Figs. 5a–5c.

### 2.4.2 Brown Pigment in Parasite

The brown pigment in the trophozoite stage of the malaria parasite only exists in PK species. The hue, saturation and value (HSV) color model [25] is applied to the segmented RBC images as in Figs. 6a–6c. Hence, HSV threshold value is set as (0.00, 0.59, 0.51). The brown pigment detection in the HSV color model is shown in Figs. 6d–6f. On the other hand, Fig. 7 shows the detail of brown pigment detected in PK.

### 2.4.3 Texture Feature Using GLCM Technique of Infected RBC

The GLCM method [31] was used to extract the texture of grayscale infected RBC for PF, PV, and PK. GLCM characteristics such as contrast, correlation and entropy were used to extract the texture of grayscale infected RBC image. Fig. 8 shows the grayscale images that have been used to extract the texture features of infected RBC.

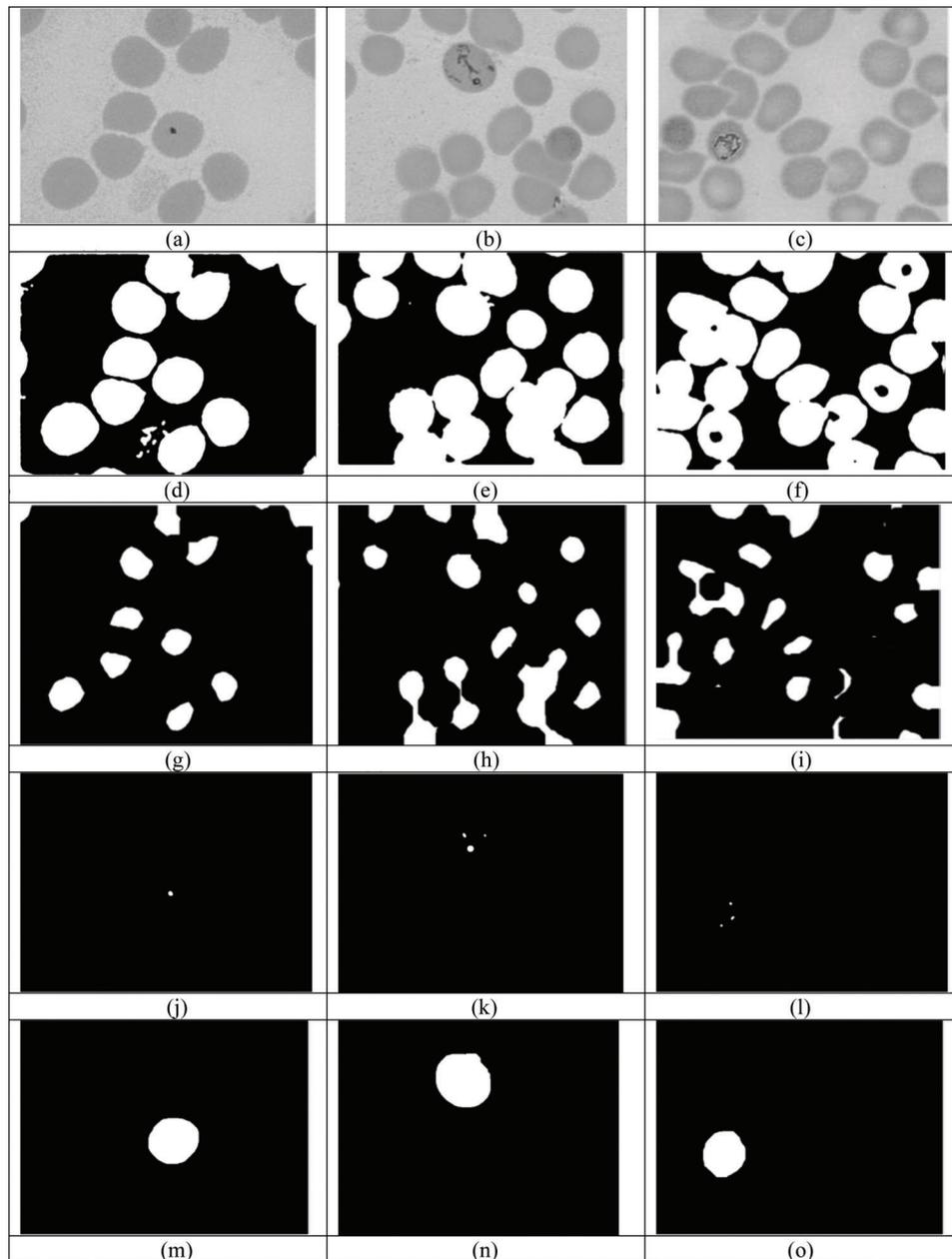
## 2.5 Classification

All data from the feature extraction process has been analysed using Multilayer Perceptron (MLP) for the classification process. In this study, the Levenberg-Marquardt (LM), Bayesian Regulation (BR) and Conjugate Gradient Backpropagation (CGP) had been used as a learning algorithm to train and test for the accuracy and robustness of the result [32]. A total of 150 images were divided into 90% for training and 10% for testing datasets.

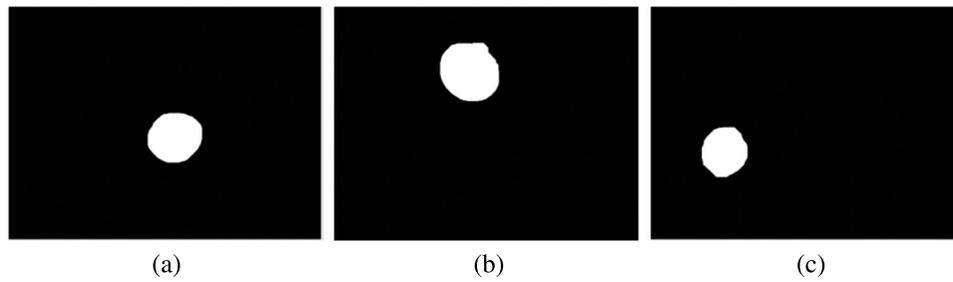
Tab. 1 shows the comparison performances of the three classifiers. BR provided the highest classification accuracy of 99.81% for training and 98.95% for testing. Therefore, the proposed procedure can effectively differentiate the trophozoite stages between PF, PV and PK images and has produced promising results in trophozoite stages of malaria.

## 2.6 Graphical User Interface for Malaria Classification System

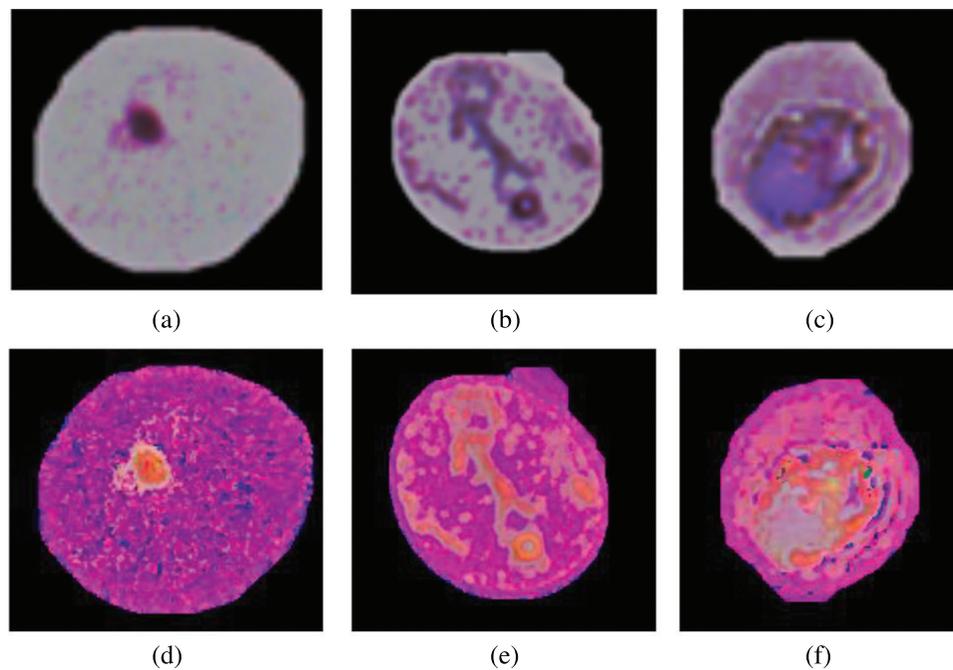
Fig. 9 shows the graphical user interface (GUI) implemented inside the proposed intelligent classification system for trophozoite stages in PK, PF and PV. To perform the malaria classification process, the user must first select the blood sample image by clicking the 'LOAD IMAGE' button. The image will display in the 'Input Image' layout, as shown in Fig. 9. Then, the user starts the automatic image processing and classification by clicking the 'PROCESS IMAGE' button. The processed image will display in the 'Processed Image' layout (Fig. 9). Finally, the classification result will show in the status box labelled as 'Result'.



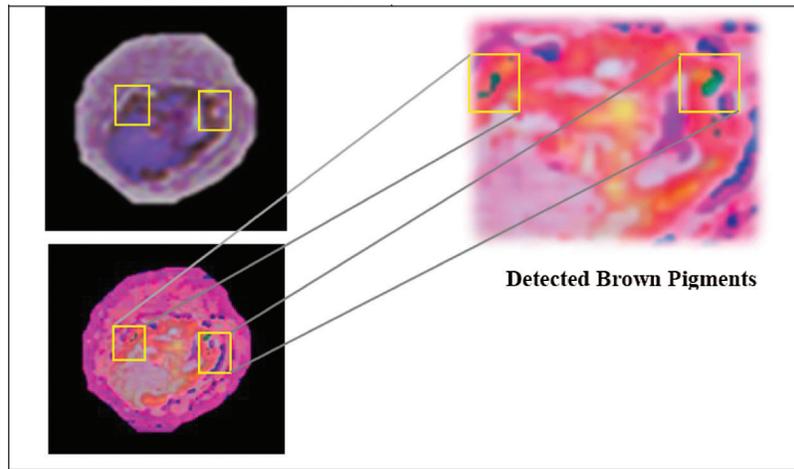
**Figure 4:** The segmentation procedure for trophozoite stages in PF, PV and PK malaria species. (a) Grayscale PF image (b) Grayscale PV image (c) Grayscale PK image (d) Result of Otsu's method for PF (e) Result of Otsu's method for PV (f) Result of Otsu's method for PK (g) Result of erosion for PF (h) Result of erosion for PV (i) Result of erosion for PK (j) Result of PF coordinate (k) Result of PV coordinate (l) Result of PK coordinate (m) Result of PF infected cell (n) Result of PV infected cell (o) Result of PK infected cell



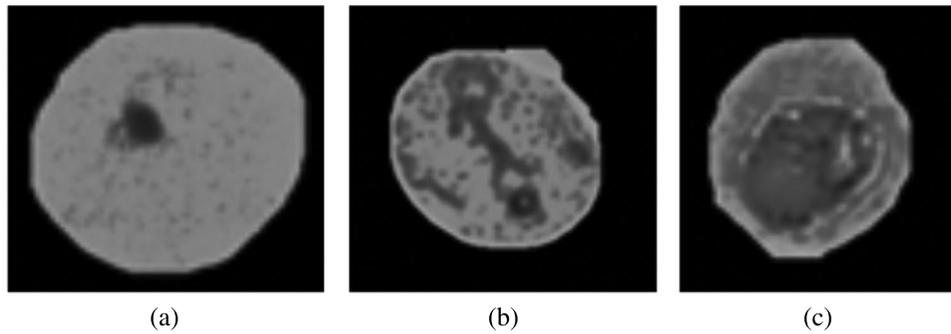
**Figure 5:** Feature extraction for the size of infected RBC (a) Binary image of PF (b) Binary image of PV (c) Binary image of PK



**Figure 6:** The brown pigment detection in HSV color model (a) Color Segmented PF image (b) Color Segmented PV image (c) Color Segmented PK image (d) HSV PF image (e) HSV PV image (f) HSV PK image



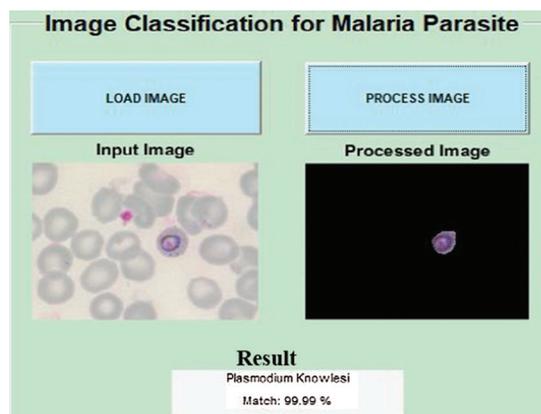
**Figure 7:** Detection of brown pigment in PK



**Figure 8:** Feature extraction for texture analysis (a) Grayscale PF image (b) Grayscale PV image (c) Grayscale PK image

**Table 1:** The result using a different type of training and testing datasets

Training algorithm	Training data (90%)	Testing data (10%)
<b>LM</b>	97.54	96.35
<b>BR</b>	<b>99.81</b>	<b>98.95</b>
<b>CGP</b>	94.37	92.94



**Figure 9:** The GUI of an Intelligent classification for Malaria parasite

### 3 Conclusion

An Intelligent Classification System for Trophozoite Stages in PK, PF and PV Malaria Species has been successfully analysed using image processing and classified using MLP to get the performance of the data. In the image processing method, the process of contrast enhancement, proposed segmentation procedure and feature extraction is used to obtain image characteristics. The experimental results have been performed using MLP-BR to obtain the good accuracy of classification. The proposed system helps classify trophozoite stages of PK, PF and PV with an accuracy of 98.95%.

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**Conflicts of Interest:** The authors declare that they have no conflicts of interest to report regarding the present study.

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