



Plasmodium falciparum Identification Using Otsu Thresholding Segmentation Method Based on Microscopic Blood Image

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Abstract.

Purpose: Malaria, particularly the *Falciparum* strain, is a deadly disease caused by *Plasmodium falciparum*. Malaria diagnosis heavily relies on a microscopist's expertise. To expedite identification, machine learning research has been extensively conducted. However, it has not yet achieved high accuracy, partly due to the use of microscopic images as the dataset. Otsu thresholding, an optimal image segmentation method, maximizes pixel variance to separate the foreground (objects of interest) from the background, which is especially effective in various lighting conditions. Otsu thresholding aims to enhance accuracy and reliability in detecting and classifying *Plasmodium falciparum* parasites in blood samples.

Methods: Dataset of microscopic images of thin blood smears with *Plasmodium falciparum*, taken from several sources, such as the UC Irvine Machine Learning Repository (UCI), National Institutes of Health (NIH), Kaggle, and Public Health Image Library (PHIL). The methodology combines various image processing techniques, including illumination correction, contrast enhancement, and noise filtering, to prepare the images effectively. The subsequent segmentation using Otsu thresholding method isolates the parasite regions of interest. The classification process involving CNN and SVM evaluates the performance of accuracy to identify different stages of *Plasmodium falciparum* parasites.

Results: The research found the effectiveness of the Otsu thresholding segmentation method in identifying *Plasmodium falciparum* parasites in microscopic images. By utilizing Convolutional Neural Network (CNN) and Support Vector Machine (SVM) classifiers, the study achieved impressive accuracy rates, with the CNN achieving 96.5% accuracy and the SVM achieving 95.7%.

Novelty: This research recorded significant improvement over previous studies that utilized feature extraction and selection methods. At the same time, previous research achieved an accuracy of 82.67. The key innovation here is the adoption of the Otsu thresholding segmentation method, which enhances the identification of *Plasmodium falciparum* parasites in microscopic images by integrating traditional image processing techniques like Otsu thresholding with modern machine learning methods like CNN and SVM. This significant improvement suggests that the proposed approach offers a more robust and reliable solution for malaria diagnosis.

Keywords: Image processing, Otsu thresholding, Classification

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INTRODUCTION

Malaria, a vector-borne infectious disease caused by *Plasmodium* parasites, continues to pose a significant global health challenge, particularly in regions with limited healthcare resources. Among the various *Plasmodium* species, *Plasmodium falciparum* stands out as the most virulent, responsible for the majority of severe malaria cases and fatalities worldwide [1]. A timely and accurate diagnosis of *P. falciparum* infection is pivotal for effective patient management, targeted treatment, and the mitigation of its life-threatening complications [2]-[3]. Microscopic examination of thick and thin blood smears has long been the gold standard for malaria diagnosis, allowing for the direct visualization of the parasites and the determination of their species and developmental stages. However, this traditional method, while highly reliable when performed by experienced microscopists, is labor-intensive and dependent on human

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expertise, making it susceptible to errors, especially in resource-limited settings. Research on malaria identification using machine learning methods has been widely conducted to expedite the identification process.

However, due to the use of microscopic image datasets, the image preprocessing stage becomes crucial in improving accuracy performance. Image segmentation is one of the common image preprocessing techniques. Image segmentation techniques consist of three types: edge-based, region-based, and pixel-based. A commonly used segmentation technique for microscopic images is thresholding. Previous research utilized thresholding based on the saturation histogram method. It involves the analysis of the saturation component of an image in the HSV (Hue, Saturation, Value) color space. The saturation component represents the intensity or purity of colors in the image [1s], [2], [4], [5]. In recent years, advances in digital image processing and computer-aided diagnostics have offered a promising avenue to enhance the accuracy [6] and efficiency of malaria diagnosis [7]. Among these techniques, the Otsu thresholding segmentation method has emerged as a valuable tool for the automated identification of *Plasmodium* parasites within microscopic images of blood smears [5], [8], [9]. By leveraging image analysis algorithms, this method seeks to delineate parasites from surrounding blood components, facilitating their precise identification and classification [10].

This article presents a comprehensive exploration of the "*Plasmodium falciparum* Identification Using Otsu Thresholding Segmentation Method Based on Microscopic Image". We delve into the rationale behind employing Otsu thresholding as an image-processing technique and its potential to revolutionize the field of malaria diagnosis. Malaria parasite detection using deep learning techniques has been done several times. They have used a pre-trained CNN architecture called InceptionV3 to extract discriminative features from a dataset of thin blood smear images [11]–[13]. These features are then used to train a machine-learning classifier to distinguish between images containing infected and non-infected malaria parasites.

The study demonstrates that the CNN-based feature extraction approach significantly improves the accuracy of 95.9% of malaria parasite detection compared to traditional handcrafted feature extraction methods, but InceptionV3 may carry biases from the data they are originally trained on. These biases could impact the model's performance, especially when applied to different demographic groups or regions [14]. Deep learning architectures like CNN (Convolutional Neural Networks) typically yield good performance in terms of accuracy [15]. However, they often require a significant amount of time for processing. Therefore, in the processing of machine learning models that use CNN architectures, it is common to perform image preprocessing and image segmentation first before proceeding to the classification stage. Resizing the image, applying noise filters, and enhancing the image are parts of image preprocessing.

Meanwhile, image segmentation techniques include some methods, such as thresholding, clustering, or watershed. In the image segmentation process, the Otsu thresholding method is frequently used since it is an effective and simple technique for automatically determining an optimal threshold to separate objects or regions of interest from the background in an image [4], [9], [16]–[21]. In previous research, identification of the various stages of the *Plasmodium vivax* parasite using digital microscopic images was also carried out. Feature analysis techniques were proposed to extract relevant information from the segmented parasite objects within the digital images using thresholding techniques [8], [16], [22]–[25]. This involves extracting both shape and texture features. To enhance the accuracy of stage identification, a feature selection process based on the wrapper method has been suggested. This step is crucial for identifying the most relevant features that contribute to improved classification results.

The study evaluates the performance of the proposed method using a dataset consisting of 73 digital microscopic images of *Plasmodium vivax* parasites on thin blood films. These images comprise various stages, including trophozoites, schizonts, and gametocytes. Performance metrics such as accuracy, sensitivity, and specificity are used to assess the effectiveness of the classification approach, and the results reached 97.29% accuracy. The article primarily focuses on *Plasmodium vivax* parasites with a small dataset, and the methods employed may not be directly transferable to other *Plasmodium* species or different parasitic infections [26]. Furthermore, an intelligent classification system designed to identify trophozoite stages in malaria species using image processing techniques and machine learning algorithms, such as Multilayer Perceptron (MLP) [4], [5], [20], [24], [26] and trained using Bayesian Rules (BR) reached the accuracy of 98.95%, higher than that of the Levenberg Marquardt (LM) and Conjugate Gradient Backpropagation (CGP) algorithm. Although they reached the higher accuracy, deep learning models or

complex machine learning algorithms might lack interpretability if applied to small datasets [27]. In an era marked by the convergence of medical science and computational technology, the development of automated malaria identification systems holds great promise for expediting diagnosis, particularly in regions where the malaria burden is substantial, and healthcare resources are constrained. This article represents a critical step toward harnessing the potential of digital image processing to revolutionize malaria diagnosis, ultimately contributing to more effective disease management and improved patient outcomes.

METHODS

The methodology comprises three primary stages: preprocessing, segmentation, and performing classification, as illustrated in Figure 1. Preprocessing techniques are applied to the images to enhance their quality and prepare them for further analysis [11], [22], [28]–[31]. This may include resizing the images, noise reduction, contrast adjustment, and other enhancements. The segmentation process uses Otsu thresholding method, which is a key component of the study. It automatically determines an optimal threshold to segment the images into foreground (parasite) and background (blood cells) regions [5], [8], [9], [32]. This threshold is chosen to maximize the variance between the two classes. The last step in this research is to classify *Plasmodium falciparum* using Convolutional Neural Network (CNN) and Support Vector Machine (SVM) to determine the effectiveness and reliability of the models.

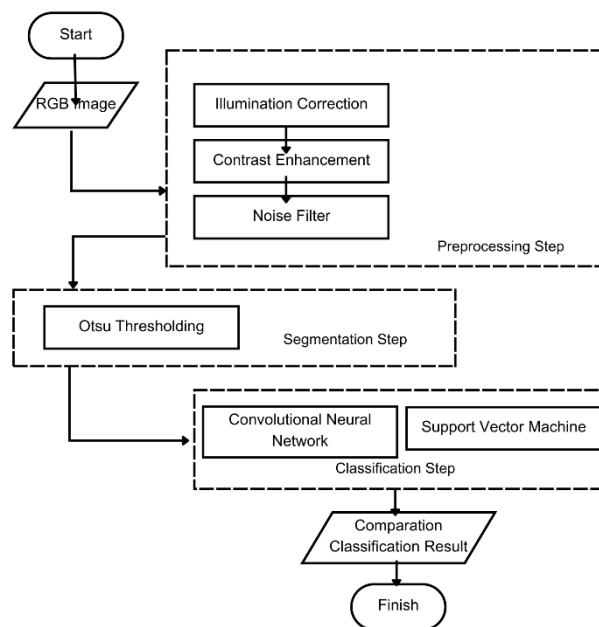


Figure 1. The proposed models for the classification of *Plasmodium falciparum*

The study began by collecting a dataset of microscopic images of thin blood smears containing *Plasmodium falciparum* parasites. These images served as the primary data for the analysis. The research dataset used public datasets taken from several sources, such as the UC Irvine Machine Learning Repository (UCI), National Institutes of Health (NIH), Kaggle, and Public Health Image Library (PHIL). Sample data collection is shown in Figure 2. The image has a resolution of 2592×1944 pixels and a color depth of 24 bits [22]. Figure 3 illustrates the process of cropping from the original thin blood smear image depicted in Figure 3(a) to focus on the region of interest (RoI) containing an infected red blood cell (RBC) affected by *Plasmodium falciparum*, resulting in a resolution of 250×250 pixels as shown in Figure 3(b).

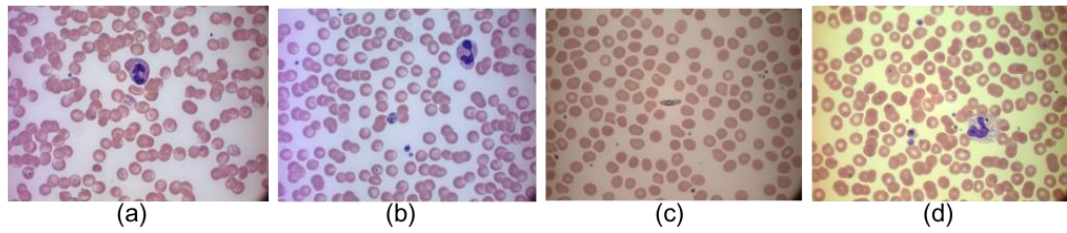


Figure 2. Sample dataset of microscopic images of thin blood smears contain *Plasmodium falciparum* (a) ring, (b) schizont, (c) gametocyte, and (d) trophozoite

After the cropping process, a total of 1.667 images were acquired. These images comprised 1.279 samples of *Plasmodium falciparum* at the ring stage, 65 schizonts, 258 trophozoites, and 65 gametocytes. Due to the occurrence of imbalanced data, to obtain accurate research results, only 65 images were selected for each stage. Figure 4 displays representative images from each of these stages: Figure 4(a) shows the ring stage, (b) features schizont, (c) gametocyte, and (d) illustrates trophozoite.

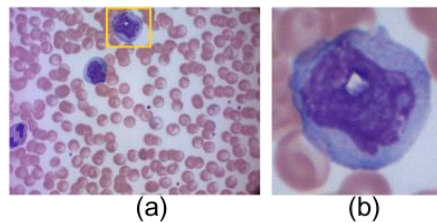


Figure 3. (A) Illustrating the cropping process and (b) showing the region of interest (ROI) infected *Plasmodium falciparum*

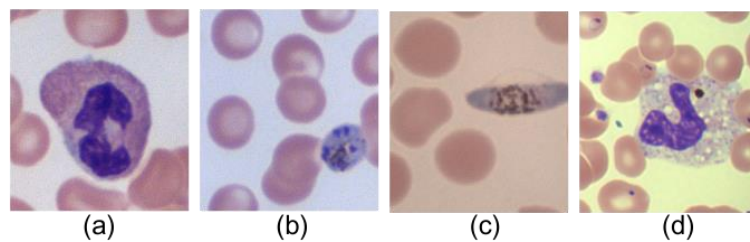


Figure 4. (a) Ring stage, (b) schizont, (c) gametocyte, (d) trophozoite

Figures of the *Plasmodium falciparum* stage in Figure 4 show uneven or non-uniform illumination. This occurs when certain areas of an image are brighter or darker than others due to various factors such as uneven lighting conditions, lens vignetting, or optical imperfections [23]. To solve the problem, illumination correction was conducted to normalize the illumination across the entire image and make it more consistent. This correction enhances the visibility of details and ensures that image analysis algorithms, such as object detection or segmentation, operate on a more even playing field. Retinex algorithms aim to estimate and remove the illumination component from an image by analyzing the color and brightness information. This can be particularly useful for color images. The Retinex algorithm can be described as follows [33], [34].

$$O = R \blacksquare I \quad (1)$$

In this context, O denotes the observed image, while R corresponds to the image's reflectance and illumination, respectively. The multiplication is denoted by the operator " \blacksquare ". In this article, we employ a logarithmic transformation to simplify the computational workload. This leads us to the subsequent expression.

$$\log(O) = \log(R \blacksquare I) \quad (2)$$

Ultimately, Equation (3) can be derived for estimating the reflectance within the HSV color space.

$$\log(R) = \log(O) - \log(I) \quad (3)$$

The next stage in image preprocessing is contrast enhancement. It is a fundamental image processing technique used to improve the visual quality and perceptibility of details in an image. It aims to increase the differentiation between the dark and light areas of an image, thereby enhancing its overall appearance [35]. One of the common techniques for contrast enhancement is Histogram Equalization. Histogram equalization is a widely used method that redistributes the pixel intensities in an image to create a more uniform histogram. It enhances the contrast by stretching the intensity range [27], [35], [36]. Figure 5 shows the steps of Histogram Equalization.

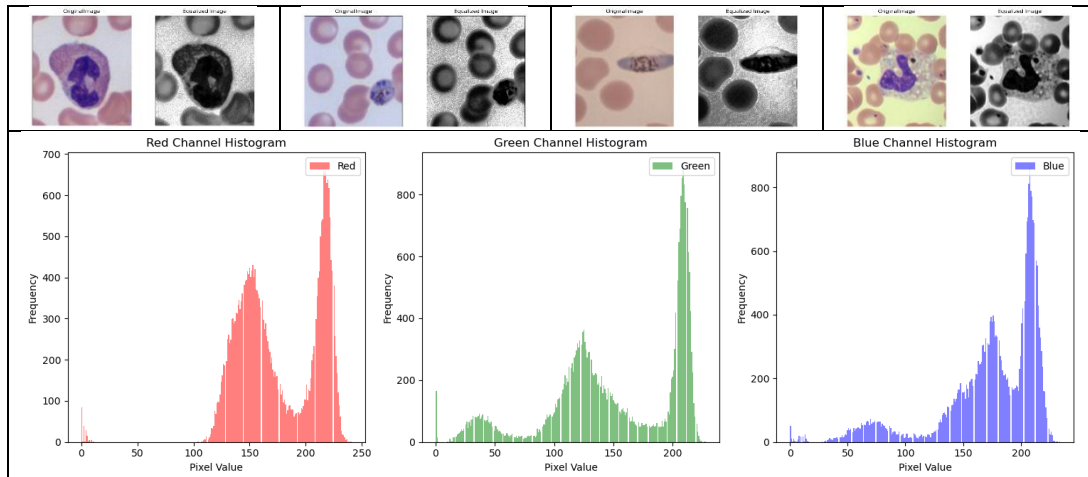


Figure 5. The histogram equalization of ring, schizont, gametocyte, and trophozoite

The last step of the preprocessing step is noise filtering using the Gaussian method aimed at reducing or removing unwanted and undesirable artifacts or variations in an image, referred to as "noise." Noise can be caused by several factors, including electronic interference, sensor limitations, environmental conditions, or even inherent characteristics of the imaging process. The goal of noise filtering is to enhance the image's quality, making it more suitable for analysis, interpretation, or visualization [21], [37].

Otsu thresholding is an image segmentation method used to automatically determine an optimal threshold for separating objects or regions of interest from the background in a grayscale image. Otsu thresholding aims to maximize the variance between two classes of pixels: foreground (objects of interest) and background. By finding the threshold that maximizes this variance, the method effectively separates the two classes [5], [8], [9]. Figure 6 illustrates Otsu thresholding step in the *Plasmodium falciparum* stage.

$$b_1(t) = \sum_{i=1}^t P(i) \quad (4)$$

P : Probability
i : Image Pixel
 $b_1(t)$: Weight Background Class 1
 $b_2(t)$: Weight Background Class 2
L : Graylevel

The mean class of the background and foreground is formulated as follows:

$$m_1(t) = \sum_{i=1}^t i \cdot P(i) / b_1(t) \quad (5)$$

$$m_2(t) = \sum_{i=1}^t i \cdot P(i) / b_2(t) \quad (6)$$

$m_1(t)$: Mean class background

$m_2(t)$: Mean class foreground
 $b_1(t)$: Weight Background Class 1
 $b_2(t)$: Weight Background Class 2
 P : Probability
 i : Image Pixel

To achieve the objectives of the Otsu method, maximizing the variance weight between classes is the right solution. To calculate the variance between classes, the following equation is used:

$$\sigma_B^2(t) = b_1 \cdot [m_1(t) - m_2(t)]^2 + b_2 \cdot [m_2(t) - m_1(t)]^2 \quad (7)$$

$\sigma_B^2(t)$: Between-Class Variance
 $m_1(t)$: Mean class background
 $m_2(t)$: Mean class foreground
 $b_1(t)$: Weight Background Class 1
 $b_2(t)$: Weight Background Class 2

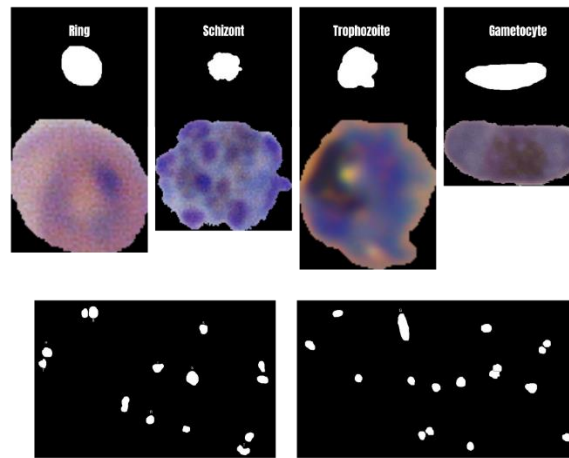


Figure 6. Illustration of otsu thresholding step in the *Plasmodium falciparum* stage.

The final stage of this research is to classify images using the Convolutional Neural Network (CNN) and Support Vector Machine (SVM) methods. CNN is one of the deep learning classification methods that has the potential for high-accuracy results [38]–[41]. CNN consists of three main layers: "convolution", "pooling", and "fully connected". Figure 7 illustrates the CNN algorithm.

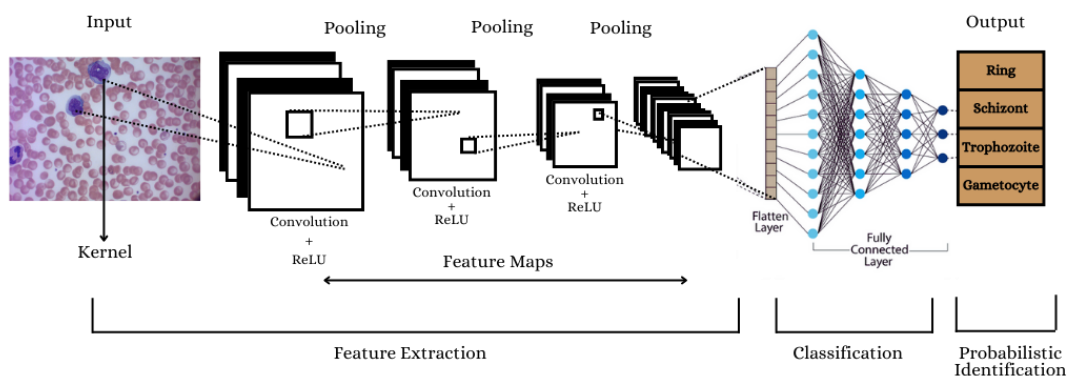


Figure 7. Convolutional neural network (CNN) architectures

SVM classifiers (linear and RBF) are currently applied within the detection framework due to their exceptional generalization capabilities and reputation for achieving high accuracy in training datasets. This method is based on statistical learning theory and the principle of structural risk minimization [42]. The

classification strategy aims to find the optimal separating hyperplane with the maximum margin between classes, focusing on the training samples located at the edges of the class distribution. The system's performance will be evaluated using a Confusion Matrix to obtain accuracy. Below is the equation for measuring the effectiveness of the system [43].

$$Accuracy = \frac{TP + TN}{TP + FP + TN + FN} \tag{8}$$

RESULTS AND DISCUSSIONS

In the research process, meticulous attention was given to data selection, dataset splitting, classification, and evaluation to identify *Plasmodium falciparum* parasite stages in microscopic images effectively. Data selection involved carefully choosing 65 images for each parasite stage from an initial dataset of 1,667 images to address potential imbalances and ensure research accuracy. This step aimed to create a well-balanced representation of different stages for subsequent analysis. Figure 8. shows the distribution of malaria staging data (ring, trophozoite, schizont, and gametocyte).

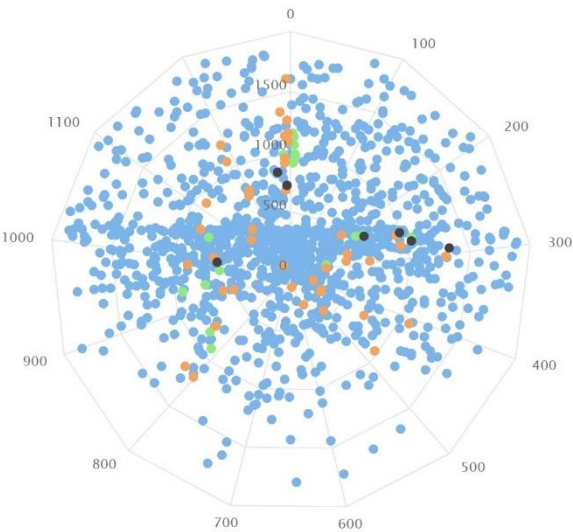


Figure 8. Distribution of malaria stage (ring, trophozoite, schizont, and gametocyte)

Subsequently, the dataset was split into training and testing subsets, with 60% allocated for training and 40% for testing. This division allowed machine learning models, including Convolutional Neural Network (CNN) and Support Vector Machine (SVM) classifiers, to be trained on one portion and evaluated on another. The performance of these classifiers was rigorously assessed using various metrics, such as accuracy, to gauge their effectiveness in accurately classifying parasite stages in microscopic images. This systematic approach ensured reliable and robust results in the research, ultimately yielding impressive accuracy rates. The performance result of the proposed method is described in Table 1.

The combination of well-considered data selection, dataset splitting, classification, and comprehensive evaluation led to the successful identification of different *Plasmodium falciparum* parasite stages. This research methodology offers a promising solution for malaria diagnosis and parasite identification, with the potential to assist healthcare professionals in providing timely and accurate patient care [20].

Table 1. Performance result of the proposed method

Identification Object	Otsu Thresholding + CNN				Otsu Thresholding + SVM			
	Accuracy	Standard Deviation	Gains	Total Time	Accuracy	Standard Deviation	Gains	Total Time
<i>P. falciparum</i> Image Segmentation (Proposed)	96.5%	±1.2%	6	5s	95.7%	±1.5%	0	14s

The proposed method in this research demonstrates an accuracy rate of 95.7% for identifying *Plasmodium falciparum* stages (ring, trophozoite, schizont, and gametocyte) using the Support Vector Machine (SVM)

classification method, while deep learning classification, namely CNN, achieves an accuracy of 96.5%. The comparison of the proposed method's performance with previous research lies in the accuracy level. In previous research, which utilized feature selection methods, the accuracy rates were 72% for texture-based feature selection, 76% for morphology-based feature selection, and 82.67% for a combination of texture and morphology-based feature selection [22]. This signifies the success of this study in applying Otsu thresholding in the preprocessing stage and classification using convolutional neural networks. Performance Comparison Proposed Method and Previous Research is shown in Table 2.

Table 2. Performance comparison proposed method and previous research

Accuracy (%)				
Features Selection (based on texture) [22]	Features Selection (based on Morphological) [22]	Features Selection (texture+morphological) [22]	Image Segmentation (Otsu+SVM)	Image Segmentation (Otsu+CNN) proposed
72%	76%	82.67%	95.7%	96.5%

CONCLUSION

This research demonstrates the effectiveness of the Otsu thresholding segmentation method in identifying *Plasmodium falciparum* parasites in microscopic images. The study employed both Convolutional Neural Network (CNN) and Support Vector Machine (SVM) classifiers for classification tasks. The results are highly promising, with CNN achieving an accuracy of 96.5% and the SVM achieving an accuracy of 95.7%. These high accuracy rates indicate the robustness and reliability of the proposed methodology in differentiating and classifying parasite-infected regions accurately. This research not only contributes to the field of malaria diagnosis and parasitic identification but also underscores the potential of combining traditional image processing techniques like Otsu thresholding with modern machine learning classifiers like CNN and SVM to improve the accuracy and efficiency of medical image analysis. The outcomes of this study hold significant promise for the development of automated systems to assist healthcare professionals in the rapid and accurate diagnosis of malaria, ultimately contributing to improved patient care and the global effort to combat this disease. Further research and validation on larger and more diverse datasets could enhance the applicability of this methodology in real-world clinical settings.

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