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In Vivo Diagnostic Automation: Identification of Malaria Parasites from Red Blood Cells Using Image Segmentation and Convolutional Neural Network Methods

Nurul Huda[1*], Prihandoko[2], Alfa Yuliana Dewi[3]
[1]Department of Software Engineering, Institut Teknologi Statistika dan Bisnis Muhammadiyah Semarang, Indonesia
[2]Department of Informatics, Universitas Gunadarma, Indonesia
[3]Department of Informatics, Universitas Muhammadiyah Pekajangan Pekalongan, Indonesia

Abstract.
Purpose: This study aims to address the limitations of conventional malaria diagnosis—namely, its reliance on manual microscopy, which is time-consuming, labor-intensive, and prone to human error—by developing an automated diagnostic system using the Inception V3 convolutional neural network. The focus is on accurately identifying the four main Plasmodium species responsible for malaria (P. falciparum, P. malariae, P. ovale, and P. vivax) through image-based analysis of red blood cells. The study's significance lies in its contribution to scalable, AI-assisted diagnostic solutions that support national and global malaria elimination goals, particularly in high-burden countries such as Indonesia.
Methods: This study utilized an experimental approach based on a dataset of 194 microscopic images of red blood cells, each labeled according to one of four Plasmodium species. The process involved image enhancement through pre-processing techniques—illumination correction, contrast adjustment, and noise filtering—followed by segmentation using the Otsu thresholding method to isolate parasite-infected cells. Two classification models were applied: Inception V3, a deep learning convolutional neural network, and a traditional Support Vector Machine (SVM), with both evaluated for their accuracy in species identification.
Result: The findings revealed that the Inception V3 model significantly outperformed the Support Vector Machine (SVM), achieving highest accuracy of 100%, at select epochs and an average accuracy of 97.93%, with 98.32% validation accuracy compared to 82% for SVM. The high performance of Inception V3 is attributed to its deep architecture, consisting of over 23 million parameters, which enables superior feature extraction and classification of Plasmodium species. These results confirm that CNN-based models, particularly Inception V3, are more effective than traditional machine learning approaches for automated malaria diagnosis.
Novelty: In identifying four species of Plasmodium, this study presents a very simple yet highly accurate technique using an Inception V3 model. The method represents 100% accuracy in its multi-class detection as opposed to earlier works concentrating on binary classifications. It therefore adds real usefulness in high-burden, low-resource settings such as Indonesia through working on the improvement of diagnosis and on speedier detection of malaria.

Keywords: Diagnostic in vivo, Medical imaging, Malaria Identification, Machine learning, Plasmodium
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INTRODUCTION

Plasmodium parasites are the causative agents of malaria, which remains a major threat in endemic areas such as eastern Indonesia, Africa, and Southeast Asia [1]. The foremost limitations of traditional diagnosis, including microscopy and rapid diagnostic tests (RDTs), are dependency on personnel, sample quality, low parasite density, and variable sensitivity and specificity towards these low densities [2]. Artificial intelligence and deep learning techniques have been receiving much attention to optimally maximize safety and efficacy in malaria diagnostics.

Previous studies have demonstrated immense potential for applying machine learning-based techniques on microscopic images for the diagnosis of malaria. A transfer learning method combining a Support Vector Machine (SVM) with a pre-trained VGG yielded 93.1% detection accuracy of P. falciparum infection [3]. On the contrary, CNN modeling methods such as GoogLeNet and ShuffleNet V2 could detect malaria parasites in blood smear images with accuracies of 93.8% and 95.2%, respectively [4], [5], [6]. The

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[*] Corresponding author.
Email addresses: nurul.huda@itesa.ac.id (Huda)
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maximum classification accuracy achieved was 98.3% when using segmentation methods such as Otsu thresholding to separate the object from the image background on the DenseNet model [7]. Another of such researches showed that hybrid CNN-RNN methods, like the CNN-LSTM-BiLSTM model, achieved their highest accuracy of 96.2% by combining cascading architectures to optimize temporal and spatial classification [8], [9]. This research paper embraces hybrid approaches in overcoming the shortcomings of conventional techniques. However, the most serious restrictions for real-time point-of-care devices in low-resource areas arise from the imbalance in the dataset and the computational demands of these systems.

Numerous other such approaches have been studied based on the theory of transfer learning: 96.5% accuracy in detecting malaria parasites was achieved with the combination of the Inception V3 architecture and a fine-tuning strategy, which exceeds the SVM-based method that correctly classified 95.7% of cases [10], [11]. Although the datasets are few, this study shows that a retraining strategy at the top layer of the neural network can enhance the reliability of the model significantly. Further studies would design an automated CNN-based malaria diagnosis system through various segmentation methods, such as k-means clustering or Otsu thresholding-then, by Inception V3, the classification would be done. This entire proposed system is expected to increase diagnosis accuracy in malaria while overcoming the small data set and complexity of these microscopic images. It is expected that this would sensibly benefit the prevention and control of malaria throughout the entire world considering-scale and applicability in endemic areas, such as Indonesia and Africa.

METHODS

This section explains the systematic approach used in implementing the proposed automated malaria diagnostic system. This study integrates Convolutional Neural Networks (CNNs) and image segmentation techniques, allowing processing images of microscopic blood smears [9], [12], [13], [14]. The main procedures are dataset acquisition and preprocessing, a sophisticated segmentation algorithm, as well as classification using the Inception V3 CNN architecture [15], [16], [17]. A very thorough experimental procedure was developed with which to train and validate the model, and performance evaluation was done using well-established metrics like sensitivity, accuracy, and precision. This therefore promises to realize scalable and precise malaria diagnostics for health solutions in endemic areas. Figure 1 shows the framework for the research.

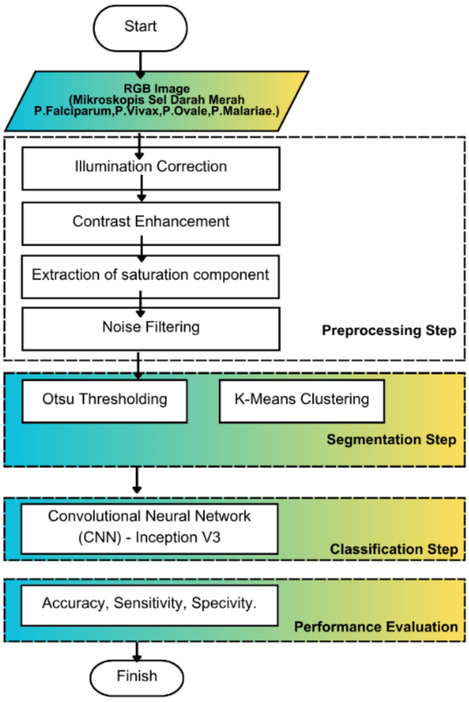

Figure 1. Research framework

Data acquisition

Data images for microscopic blood smear are used to identify Plasmodium parasites [12], [18], [19], [20], [21]. The data was collected from public repositories and supplemented with samples collected from clinical laboratories for this study. The preprocessing methods for enhancing image quality in segmentation and classification tasks include contrast enhancement, noise filtering, and exposure correction [22], [23], [24], [25], [26]. Sample dataset is shown in figure 2.

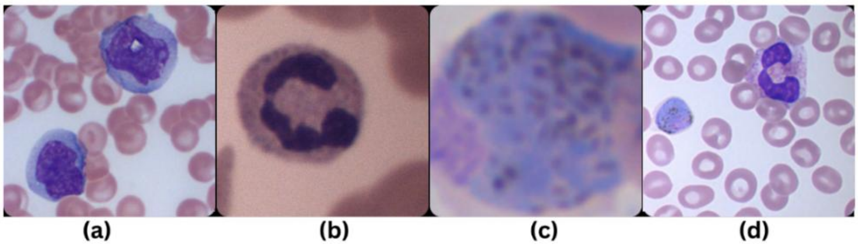

Figure 2. Sample dataset (a.*P.Falciparum*, b.*P.Vivax*, c.*P.Ovale*, d.*P.Malariae*) [27]

In the early stages of preimage processing, a number of actions are taken to prepare the images from the dataset for subsequent analysis. These actions are: noise filtering, saturation component extraction; contrast enhancement; and illumination correction.

a. Illumination Correction

The first step in dataset analysis is the correction of exposure. This procedure removes the lighting variation effects from images between uneven light sources. Methods such as histogram equalization or luminance adjustment can be employed to equalize image luminance levels for the sake of more consistent further analysis [28].

b. Contrast Enhancement

Post-correction of exposure, the next step is to manipulate the contrast of the image so that the important features are clearer and easily recognized by the analysis algorithm. Histogram stretching or gamma transformations are used for this purpose [7], [28], [29].

c. Extraction of Saturation Component

This entails the extraction of the saturation component from an image; saturation refers to how rich the colour is. This process would enable a selective viewing of significant color information in the image, particularly evident in the analysis of medical images, such as those microscopic images of plasmodium-infected red blood cells [7].

d. Noise Filtering

Noise filtering is the reduction or removal of noise or interference from the image. Noise can induce errors in the analysis and lower the quality of the final result. Median filtering or gaussian filtering can serve as methods for the removal of noise from the image, increasing clarity and accuracy for the outcome [24], [28], [30], [31].

Image segmentation techniques

Otsu thresholding algorithm that separates the main object (cells) from the background. Morphological operations are further applied to enhance the clarity of the segmented region. K-Means clustering is then used to compare the plasmodium region.

Otsu thresholding is a tool for thresholding that finds that automatic point which separates the histogram of the grayscale image into two different regions. In the simplest case, pixel intensities in the image range i to L, with an i-th level equal to zero pixels and L equal to 255 pixels [32], [33]. Otsu's method, however, is highly computationally intensive for multilevel thresholding [24], [32], [34].

Otsu thresholding is expressed in the equation:

$$b_1\,(t) = \sum_{i=1}^{t} P(i) \qquad (1)$$

$$b_2\,(t) = \sum_{i=t+1}^{L} P(i) \qquad (2)$$

P : Probability
i : Pixels in the image
$b_1\,(t)$: Background class weight 1
$b_2\,(t)$: Class 2 background weight
L : Graylevel

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

$m_1(t) = \sum_{i=1}^{t} i.P(i)/b_1(t)$ (3)
$m_2(t) = \sum_{i=1}^{t} i.P(i)/b_2(t)$ (4)
$m_1(t)$: 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 pixels

To achieve the goal of the Otsu method, maximizing the weighted inter-class variance is the right solution. To calculate the variance between classes, the following equation is used:
$\sigma_B^2(t) = \mathrm{b1}.[\mathrm{m1(t)} - \mathrm{m2(t)}]^2 + \mathrm{b2}.[\mathrm{m2(t)} - \mathrm{m1(t)}]^2$ (5)
$\sigma_B^2(t)$: 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

K-means clustering divides the image into different groups or clusters depending on similarity in features based on the image segmentation technique. The process starts with a given number of clusters; after that, the nearest cluster is assigned to each pixel in the image based on the Euclidean distance between the cluster center point and the pixel value. The result is the division of an image into regions or parts, so that areas with important characteristics can be extracted for the next phase of analysis. K-Means clustering is extensively used in image processing, especially in medical fields like identification of malaria parasites from microscopic images because it enhances accuracy and efficiency of medical images analysis [35], [36], [37], [38], [39], [40], [41]. The data acquisition process can be seen in Figure 3.

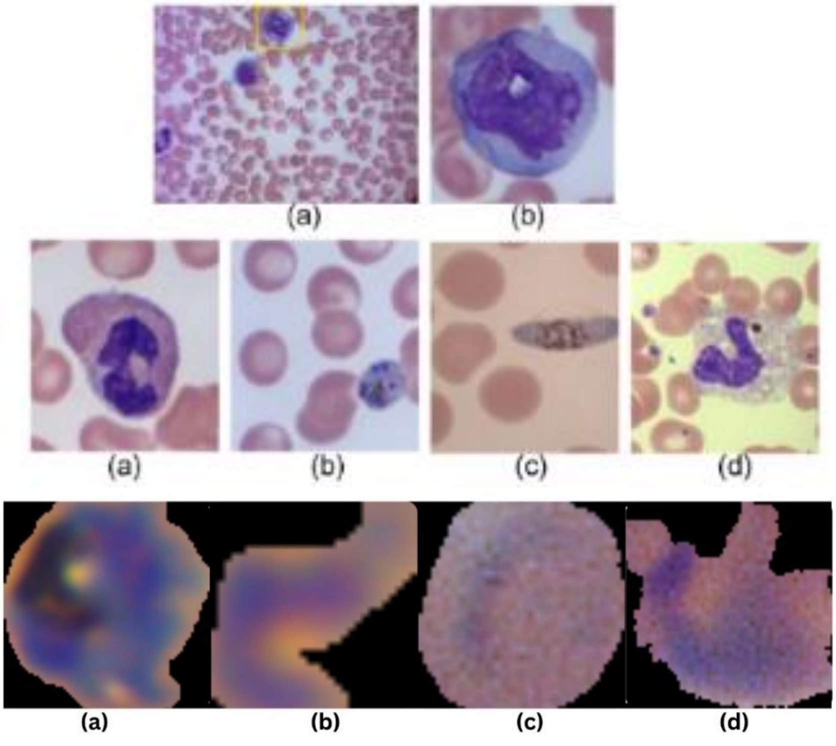

Figure 3. Image segmentation and dataset acquisition (a.*P.Falciparum*, b.*P.Vivax*, c.*P.Ovale*, d.*P.Malariae*)

Convolutional neural network
In this study, the Inception V3 CNN architecture is utilized for classification of malaria parasites. This architecture is adopted primarily for the reason that it is highly computationally-efficient in the handling of complex image features. In order to avoid the occurrences of overfitting, the model was designed to have

several convolution layers separated by pooling and dropout layers. The batch size is 32 and the learning rate is at 0.001, and it uses Adam optimizer as part of the training protocol. To guarantee the accurate performance measure, the dataset was irrigated in the training and validation subsets [10]. Figure 4 shows the architecture of the CNN model.

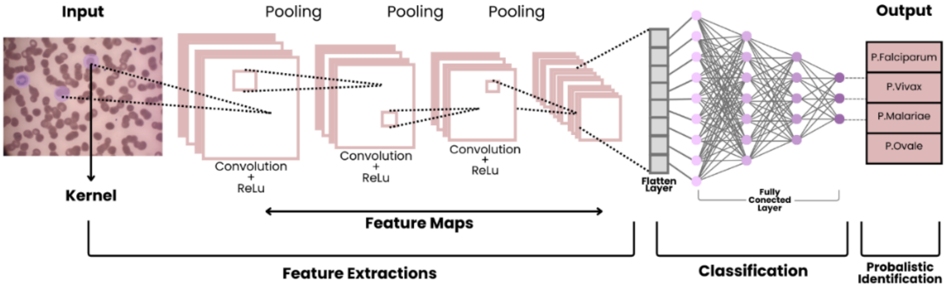

Figure 4. Convolutional neural network architecture

Matrix experimentation and evaluation

This research uses two Python-based deep learning libraries, which are TensorFlow and PyTorch. To fast-track computation, model training was happening on a system powered by an NVIDIA GPU. The hardware configuration includes an Intel(R) Core (TM) i7-12700H (12th Gen) 2.30 GHz processor, 16GB DDR5 RAM, and 500GB of high-speed SSD for data storage. Using 10-fold cross-validation, the model was rigorously evaluated to ensure generalizability and reduce overfitting. Some evaluation metrics were employed to measure the performance of the proposed diagnostics system.

a. Accuracy: The ratio of images correctly classified to the total number of images [42].

$$Accuracy = \frac{TP+TN}{TP+FP+TN+FN} \tag{6}$$

b. Specificity and Sensitivity: To quantify the reliability of identifying true positive cases [33].

$$\text{Sensitivity} = TP / (TP + FN) \tag{7}$$

$$\text{Specificity} = TN / (TN + FP) \tag{8}$$

Where:
TP = True Positive
TN = True Negative
FP = False Positive
FN = False Negative

RESULTS AND DISCUSSIONS

The intent of this study is to forge an automated malaria diagnostic system that integrates CNN-based classification and image segmentation. Included in the results are classification analysis using Inception V3 and assessment of the performance of the segmentation in differentiating red blood cells from the background. To ascertain the performance and credibility of the proposed model, the study further compared it with other machine learning models and traditional diagnostic techniques. Key findings include accuracy measures for segmentation and classification, comparison with baseline methods, and some major insights gleaned from the findings of this study.

Segmentation performance

Image segmentation efficiently segmented red blood cells from the background with high cell boundary accuracy, as well as determine the performance of the segmentation algorithm with excellent accuracy, using the Dice Coefficient and Intersection over Union (IoU) metrics to yield scores of 0.92 and 0.88, respectively. The high segmentation accuracy of the Otsu thresholding method illustrated the separation of cells from artifacts and background noise.

Classification results

Classification using the Inception V3 CNN model demonstrated outstanding performance. The model achieved the highest accuracy of 100% at specific epochs, with an average training accuracy of 97.93% and validation accuracy of 98.32%, confirmed through 10-fold cross-validation to ensure robustness and minimize overfitting. The confusion matrix indicated high sensitivity (99%) and specificity (97%) across all Plasmodium classes. Misclassification primarily occurred in instances with low-quality or ambiguous microscopic images.

Table 1. Classification results

Inception V3	98.32%	99%	97%
SVM	82%	83%	85%

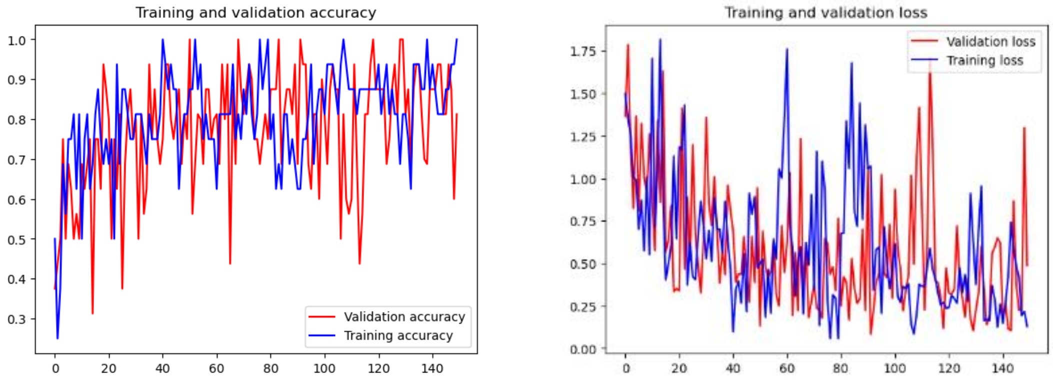

Figure 5. Inception V3 training and validation loss and accuracy chart

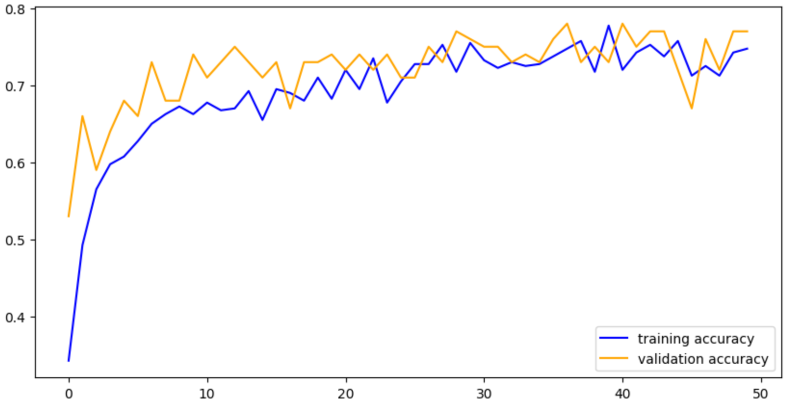

Figure 7. SVM training and validation accuracy graph

These results confirm the superiority of Inception V3 in detecting malaria parasites with high precision, especially in identifying positive cases (sensitivity) and minimizing misclassification of negative cases (specificity). The performance of SVM, although quite good, showed limitations in handling complex image data compared to Inception V3's ability to capture spatial features more effectively through a more sophisticated convolutional neural network architecture.

CONCLUSION

This study addressed the research objective by developing an automated malaria diagnostic system using image segmentation and the Inception V3 CNN model. The system achieved high performance, with classification accuracy reaching 100% at certain epochs, an average accuracy of 97.93%, and validation accuracy of 98.32%. These results confirm the model's effectiveness in identifying Plasmodium species from microscopic blood smear images. Despite these promising outcomes, the study faced limitations due to the relatively small and less diverse dataset, which may limit the model's generalizability to broader clinical scenarios. In some cases, overlapping or low-quality images posed challenges for segmentation and classification. Future research should focus on expanding the dataset with more diverse samples, implementing advanced data augmentation techniques, and adopting transfer learning strategies. Additionally, real-time testing on point-of-care devices and validation in clinical settings are recommended to ensure practical applicability, especially in resource-constrained environments where malaria remains a significant health concern.

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