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Malaria Recognition Using Cyclic Voltammetry from Light Microscopy Images as Biochemistry Application

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Abstract

Malaria is a dangerous, life-threatening disease caused by parasite called plasmodium. Plasmodium parasite transmitted to humans by the bite of infected female of *Anopheles* mosquitoes. Malaria must be accurately detected and diagnosed in early stages to treat patients and to prevent spread of malaria. Manual examination of thin blood smears is used to diagnose the disease but such examination needs expertise, specialized and well-trained people that is unfortunately limited in rural areas. Artificial intelligence (AI) and computer aided diagnostic systems (CAD) are the key solutions to develop and improve malaria's diagnosis by applying AI concept, tools and techniques on microscopic red blood cells (RBC) images. Convolutional neural network (CNN) is a class of deep learning (DL) which demonstrates promising results in image recognition and classification. In this study, a CNN model is introduced for malaria classification that applied to blood smear microscopic images help detecting weather the cell is infected or non-infected with malaria parasites. The model is applied on a dataset of segmented thin blood smear microscopic images that is provided from National Institute of Health (NIH). MATLAB 2019a software is used to build the CNN that achieves accuracy 86% and we compared this model with the pre-trained model (AlexNet) which achieves accuracy 83%, which means progress in relation to accuracy. The results demonstrated that the inclusion improved the biochemistry properties of the nanostructured prepared surface to varying degrees.

Keywords: Biochemistry, Malaria classification, Artificial intelligent, Deep learning, Convolutional neural network, Pre-trained model.

Introduction

Malaria is a fatal, life-threatening disease caused by parasite called plasmodium. Plasmodium parasite transmitted to humans by the bite of infected female of Anopheles mosquitoes that called "Malaria vectors". Five different parasites species that cause malaria in humans, which is the deadliest among these species [1]. Malaria is a widespread disease that has taken lives of millions of people worldwide. In 2018, an estimated 228 million cases of malaria occurred and 4,05,000 deaths from malaria disease all around the world. 213 million or 93% of estimated malaria cases in WHO African Region. Although it is an acute, sever and

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fatal disease but also preventable and curable. Early diagnosis with appropriate treatment can safe people lives. Microscopic Red Blood Cells (RBCs) images are commonly used for malaria diagnosis; thick and thin blood smears help detecting malaria parasites [2-5]. Manual examination of thick and thin blood smears is the most available and common method of malaria diagnosis but such examination needs expertise, specialized and well-trained people that is unfortunately limited in rural areas where malaria is dominant. In addition, lack of standardization in manual testing and the large size of microscopic blood-films develop the need of automatic diagnosis of malaria disease. (CAD) applied to blood smear microscopic images help detecting weather the cell is infected or non-infected with malaria parasites. Therefore, it has ensured accurate, correct diagnosis and assists as decision support systems with faster and reliable diagnosis. Using CAD increases the number of patients to be served by reducing both diagnosis cost and the field worker load [6-10].

Nanotechnology advancements in recent years have enabled the incorporation of novel materials into bio-analytical detection systems based on recognition elements such as enzymes, antibodies, nucleic acids, and others. Because of the selective and sensitive interaction between bio-reagents and target analytes, these devices produce simple, rapid, and accurate results without the need to remove interferants from the sample prior to detection. The use of such devices has been expanded to include process monitoring, clinical diagnosis, and the evaluation of environmental, food, and water safety. Malaria is a tropical infectious parasitic disease caused by Plasmodium sp. parasites (*Plasmodium falciparum, ovale, vivax, malariae*, and *knowlesi*) and transmitted by female *Anopheles* mosquitos. According to the World Malaria Report 2019, there were an estimated 405[thin space (1/6-em)] deaths worldwide in 2018, with Plasmodium falciparum parasite infections being the most common. Morbidity and mortality are higher in low-income populations with limited access to health care. Automated malaria examination utilizing machine-learning techniques, like deep learning, provides the promise of serving as an efficient diagnostic aid. In our work we used the AI systems to enhance the diagnosis process of malaria by using CNN on a dataset consist of segmented thin red blood smear images to classify whether the cell is infected or non-infected. We designed CNN by MATLAB software proposed, which showed good results [5:5], and the compared this model with the pre-trained model (AlexNet) which used in many researches in malaria classification. The CNN model was built from scratch with 10 layers with high sensitivity of 97% and relatively high positive predictive value (PPV) of 81%. The author also suggested a false positive (FP) reduction method utilizing feature clustering extracted from the gray level co-occurrence matrix (GLCM) from the Region of Interests (ROIs) [11-15]. The primary goal of this study was to investigate Malaria Recognition Using Cyclic Voltammetry from Light Microscopy Images as a biochemistry application. The results were comparable to those obtained using bare platinum electrodes.

Materials and Methods

Chemicals and Reagents

Sigma-Aldrich provided DHP, potassium hexacyanoferrate (III), and potassium chloride (St. Louis, USA). Merck provided the potassium hexacyanoferrate (II) trihydrate, 98% sulfuric acid, and pH 7.00 buffer saline solution (PBS) (Darmstadt, Germany). The Leônidas and Maria Deane Institute provided purified polyclonal Ab-PfHRP2, Ag-PfHRP2, and human serum samples (Oswaldo Cruz Foundation, Manaus, Brazil). The biomolecules were prepared by recombinantly expressing Plasmodium falciparum HRP2 in Escherichia coli BL21 pLysS (Invitrogen) and purifying them using affinity chromatography nickel columns (QIAGEN) according to the manufacturer's instructions. After that, mice were immunized four times with the purified recombinant protein (10 g doses every two weeks).

The reactivity of serum samples was determined using an indirect enzyme-linked immunosorbent assay (ELISA). Following the manufacturer's instructions, antibodies were purified using a protein G sepharose (Sigma) resin column. The remaining reagents were of analytical grade and were used without further purification. Purified water from a Milli-Q system was

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used to make aqueous solutions.

Data Collection

The used in our work 4000 images; 2000 image infected and 2000 images non-infected of segmented red blood cell images, as show on Figures 1 and 2.

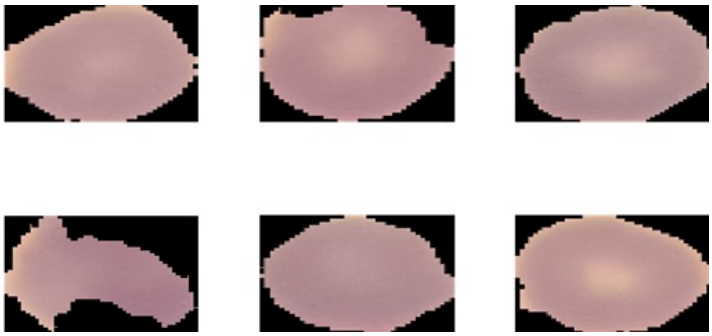

Figure 1: Samples of non-infected images

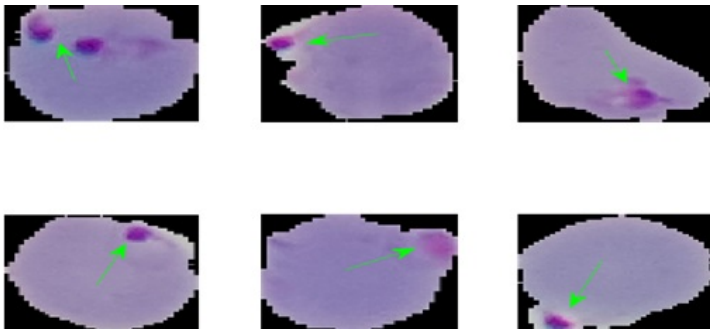

Figure 2: Samples of infected images

CNN Model Configurations

The proposed two sequential CNN architectures for classifying images to infected and non-infected with malaria parasites from RBCs thin images. We resampled the images size into 44*44 and 227*227 pixels resolution to match the input requirements of customized and pre-trained CNN (AlexNet) respectively. The two models were trained, tested and coded on a Windows®system with MATLAB® R2019a software.

Results and Discussion

A 13-layers CNN customized model is shown below in Figure 2, similar to CNN architecture proposed by Krishnan [8]. The input to the model constitutes segmented cells of 44*44*3-pixel resolution and the final layer is the Softmax classifier that measures the prediction distribution between the two classes.

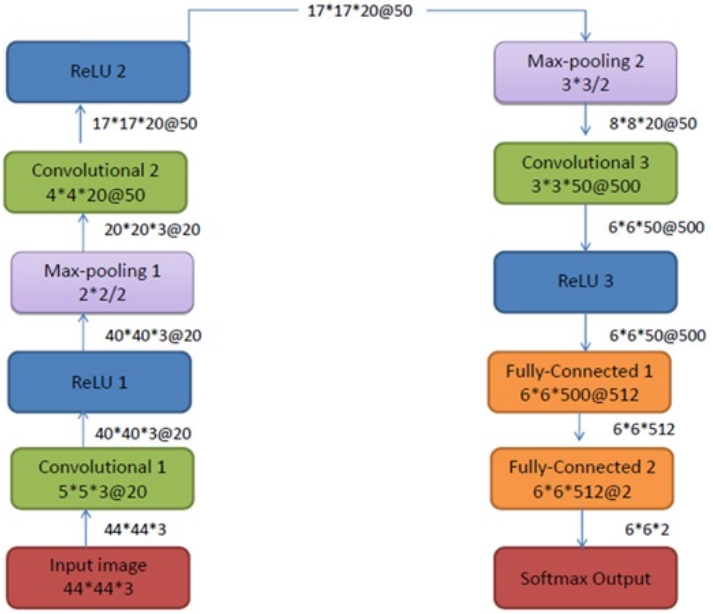

Figure 3: The customized CNN model architecture

The split the dataset into training and testing sets having the ratio 70:30 then we trained the model by using (SGD) optimizer and Nesterov's momentum. Table 1 shows a summary of the model settings along with the hyperparameter values. Finally, we evaluated the performance in terms of accuracy, sensitivity and specificity.

Parameter Name	Type / Value
Optimizer	SGD
Epochs	15
Learning rate	1.0000e-04
Momentum	0.9
Batch size	128
Input image	44*44*3

Table 1: Customized CNN Setting with Hyperparameter Values

The second proposed CNN is similar to the pre-trained AlexNet architecture consisting of 25 layers and the final layer is the Softmax classifier, which measures the prediction distribution between the two classes.

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1. 1x1 ImageInputLayer
2. 1x1 Convolution2DLayer
3. 1x1 ReLULayer
4. 1x1CrossChannelNormalizationLayer
5. 1x1 MaxPooling2DLayer
6. 1x1 GroupedConvolution2DLayer
7. 1x1 ReLULayer
8. 1x1CrossChannelNormalizationLayer
9. 1x1 MaxPooling2DLayer
10. 1x1 Convolution2DLayer
11. 1x1 ReLULayer
12. 1x1 GroupedConvolution2DLayer
13. 1x1 ReLULayer
14. 1x1 GroupedConvolution2DLayer
15. 1x1 ReLULayer
16. 1x1 MaxPooling2DLayer
17. 1x1 FullyConnectedLayer
18. 1x1 ReLULayer
19. 1x1 DropoutLayer
20. 1x1 FullyConnectedLayer
21. 1x1 ReLULayer
22. 1x1 DropoutLayer
23. 1x1 FullyConnectedLayer
24. 1x1 SoftmaxLayer
25. 1x1 ClassificationOutputLayer

Figure 4. The AlexNet CNN model architecture

The split the dataset into training and testing sets having the ratio 70:30 and we trained the model by using SGD optimizer; Table 2 shows a summary of the model settings along with the hyperparameter values. The evaluated the performance in terms of sensitivity accuracy and specificity.

Parameter Name	Type / Value
Optimizer	SGD
Epochs	5
Learning rate	1.0000e-04
Momentum	0.9
Batch size	128
Input image	227*227*3

Table 2: AlexNet CNN Setting withHyperparameter Values

In our work, we proposed two CNN networks for malaria classification form thin blood smear images. For the customized CNN model, we determined the optimum value to be 0.9 and 1.0000e-04 for the SGD momentum and learning rate, respectively while training the data with 98.44% Mini-batch accuracy 0.0766 Mini-batch loss at epoch 13. Figure 5 shows the training progress of the network and figure 6 shows the confusion matrix with 86% performance accuracy.

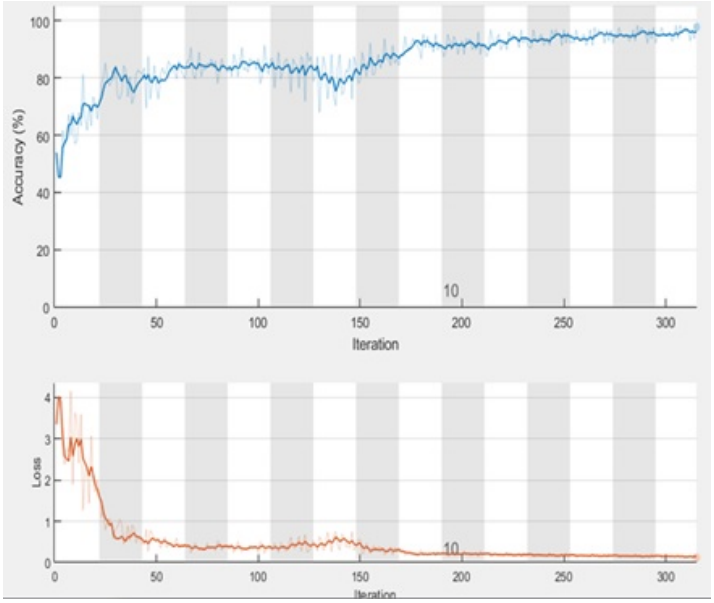

Figure 5: The training progress (Accuracy and loss) of customized CNN

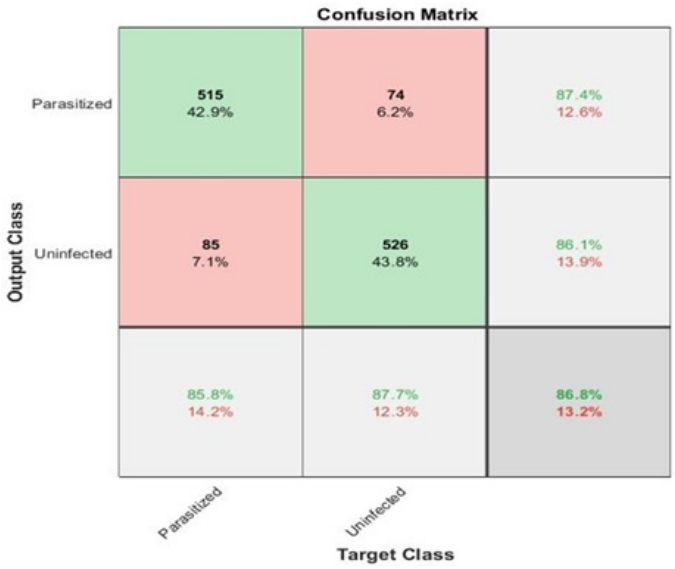

Figure 6: Confusion matrix of customized CNN

For the AlexNet CNN model, we determined the optimum value to be 0.9 and 1.0000e-04 for the SGD momentum and learning rate, respectively while training the data with 86.72% Mini-batch accuracy and 0.3681 Mini-batch loss at epoch 5. Figure 7 shows the training progress of the network and figure 8 shows the confusion matrix with 83% performance accuracy.

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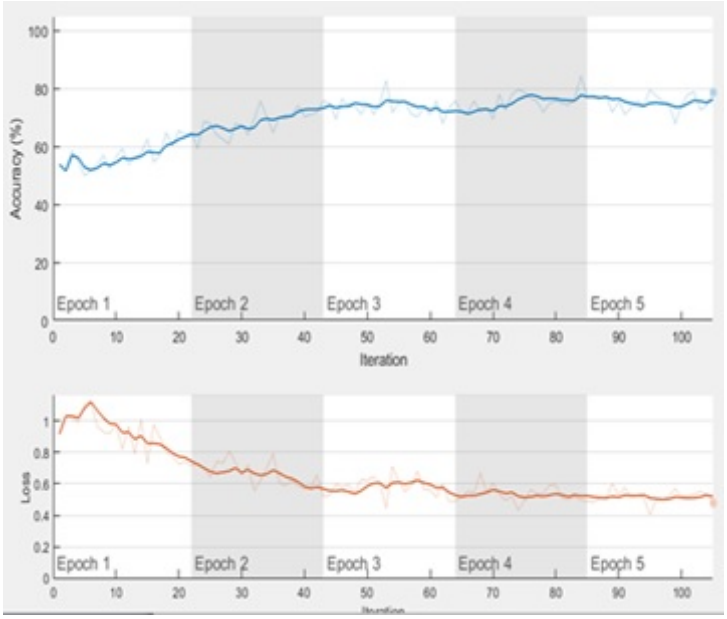

Figure 7: The training progress (Accuracy and loss) of AlexNet CNN

Figure 8: Confusion matrix of AlexNet CNN

The propose evaluation metrics for this paper are in term of Accuracy, Sensitivity and Specificity which calculated by the confusion matrix shown in Table 3.

Accuracy = (TN+TP) / (TN+TP+FN+FP)…(1)

Sensitivity =TP/ (TP+FN)… 2)

Specificity =TN/ (TN+FP)…(3)

Performance Metric	Accuracy	Sensitivity	Specificity
Customized CNN	86.8%	85.8%	87.7%
AlexNet CNN	83.5%	76.7%	90.3%

Table 3: Performance Evaluation Metrics of both CNN Models

Because of our work, the customized CNN performance is more accurate than AlexNet CNN in malaria classification that satisfies the result of, highlighting on the fact that we write both CNNs codes manually as M-file [18-21].

Conclusion

In this work, we designed manually two models of convolutional neural networks; customized CNN and AlexNet, the customized CNN achieves accuracy 86% and AlexNet achieves accuracy 83%, which means advancement in relation to accuracy. Convolutional neural network based deep learning model is extremely an excellent solution for task-specific classification like classifying the infected and non-infected cells as an effective aid in malaria examination. Deep learning models present the promise of serving as an effective diagnostic aid where manual examination can be wearisome for large-scale diagnoses. Many studies reported in the field of malaria classification and detection based on convolutional neural network and they showed good results, but also, we need more efforts to reach to the best model to get the best accuracy with minimum time and power consuming. The model is used with a dataset of segmented thin blood smear microscopic images from the National Institute of Health (NIH). The results showed that the inclusion improved the biochemistry properties of the nanostructured prepared surface to varying degrees.

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