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Classification of Overlapping Red Blood Cells Using Image Segmentation and Convolutional Neural Network

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***Abstract*: Machine vision is an analytical technique widely used to identify blood cells, considering both qualitative and quantitative attributes. Qualitative studies include differentiating red blood cells and white blood cells. Quantitative studies include counting the number of cells in a particular image. However, the problem is that the phenomenon of overlapping, especially in red blood cells images, often occurs, which causes the accuracy of image processing to be disrupted. In the present study, the image segmentation process for image red blood cells using convolutional neural network algorithms has been evaluated to predict single and overlapping classification groups on red blood cells. A total of 100 public red blood cells images were used in this study by treating 5 data splits (calibration and testing), including 80:20, 75:25, 70:30, 65:35, and 60:40. Each cell in the red blood cells image will be classified into a single red blood cells group and an overlapping red blood cells group. Probability statistical tests and ANOVA were used to obtain the best preprocessing strategy for each classification parameter, including precision, recall, F1-score, and accuracy. The results generally show that image segmentation can classify single and overlapping red blood cells with acceptable accuracy.**

Keywords: accuracy, CNN, data splitting, image segmentation, overlapping, RBC**.**

I. Introduction

Identifying and counting red blood cells is a significant health indicator as it provides valuable information about a person's overall health and well-being. Red blood cells, also known as erythrocytes, are responsible for carrying oxygen from the lungs to the rest of the body. An abnormal number of red blood cells can indicate various health conditions, such as anemia, polycythemia, and iron deficiency [1-3]. By counting red blood cells, healthcare professionals can accurately diagnose and treat these conditions, ensuring that a person's body receives enough oxygen, and their overall health is maintained.

Image processing is a non-destructive method that has recently developed, starting from the challenging engineering sector to the soft engineering sector [4-6]. Counting red blood cells (RBCs) from an image can be performed using image analysis techniques [7-9]. These techniques typically involve preprocessing the image to remove noise and enhance the visibility of RBCs, followed by segmentation to separate RBCs from the background. After segmentation, individual RBCs can be identified and counted using blob analysis, morphological operations, or machine learning algorithms. It is crucial to ensure the accuracy of the RBC count by verifying the results and adjusting the image analysis parameters as necessary. Using automated image analysis techniques for RBC counting can increase the efficiency and accuracy of the process, reducing the time and effort required for manual counting.

Non-invasive technology for recognizing and counting blood cells based on image processing has been widely developed [10-14]. This technology is expected to be less expensive and simpler to implement. This technology transforms prepared blood cells into a picture, then examines them using different image-processing techniques and procedures. In contrast to typical blood cell identification and counting methods, however, this technique is still growing to achieve optimum accuracy. Several research findings have equated performance accuracy to a hematologist's interpretation.

There are several current techniques for calculating red blood cell counts (RBCs), including manual counting, automated image analysis, and flow cytometry [15-17]. Manual counting involves microscopically examining a blood sample and manually counting the number of red blood cells, which is time-consuming and subject to observer variability. Automated image analysis uses computer algorithms to process images of blood samples and automatically count RBCs. This method can be more efficient and less subjective than manual counting. However, accuracy can be affected by variations in the shape, size, and color, as well as other cellular structures and artifacts in the image. Flow cytometry, on the other hand, uses laser-based technology to measure the physical characteristics of RBCs, such as size and granularity, and accurately count them rapidly and automated. Each of these techniques has its advantages and limitations, and the choice of method will depend on the application's specific requirements.

There is a research gap in counting red blood cells (RBCs) using image analysis techniques. Despite advances in computer vision and machine learning, there are still challenges in accurately identifying and counting RBCs in complex images [18-20]. This is in part due to variations in the shape, size, overlapping cell and color, as well as other cellular structures and artifacts in the image [21]. Furthermore, there is a lack of standardized datasets and evaluation metrics to compare different RBC counting methods, making it difficult to assess the performance of different algorithms and compare their results. To address these research gaps, more work is needed to develop robust and accurate image analysis methods for RBC counting and establish standardized data sets and evaluation metrics for comparison and validation purposes. However, to the best of our knowledge, the problem of single-cell classification and cell overlapping must be solved first before accurately calculating the number of cells. Overlapping blood cells is disturbing and noisy when applying the blood cell count algorithm [8, 22, 23]. This will significantly interfere with the accuracy of the blood cell number calculation algorithm.

The RBC classification may be stated as a pattern recognition issue [24-26]. It is among the most common RBC classifiers because of its excellent reputation. Many studies have demonstrated that neural network classification is superior to statistical pattern categorization. Significant classification efficiencies of 80% for regular and 60% for abnormal cells were observed. Nevertheless, the primary issue reported is that overcoming the overlap between red blood cells is problematic, resulting in poor accuracy when predicting the number of red blood cells in an image. Therefore, this study aims to classify single and overlapping images of red blood cells using image segmentation and convolutional neural networks.

II. Methodologies

A. RBC dataset and algorithm experimental

A total of 100 images of RBC data sets were used to evaluate the performance of the proposed method. Images of RBC were downloaded from the website https://imagebank.hematology.org/about. The American Society of Hematology has collected the data set. Researchers commonly use this data set to test the latest techniques, methods, or algorithms in the case of red blood cells [27].

Image segmentation divides an image into constituent pieces based on parameters such as pixel intensity, spectral values, and textural characteristics. Image binarization segmentation, defined as separating an image into objects and background, is the most fundamental and essential processing step and a general, primary, and essential approach to studying object recognition, image comprehension, and computer vision. The effectiveness of picture segmentation will directly affect future object recognition and visual awareness. The most straightforward and successful picture segmentation approach is based on the gray-level threshold. However, it is pretty challenging to choose a suitable point. [28-30].

Image segmentation primarily extracts three characteristics: dots, lines, and edges. Laplacian masks can be used for detecting points in photographs. Similar-shaped objects, such as single points, elicit the most significant response from these masks. The center of these masks has the most critical coefficient, whereas neighboring pixels have the opposite sign. Laplacian masks are also used to do line detection in a picture. Four available covers for line detection in various directions exist in every image. The lines are detectable in the horizontal, vertical, +45, and -45 degrees. In photos, dramatic intensity variations occur along the margins of objects. Hence, edge detection is a crucial step in picture segmentation. Using first- and second-order derivatives, the benefits of a picture are recognized [31-33].

The flowchart of the overall model structure for detecting single and overlapping groups of red blood cells in this study is shown in Figure 1. The instance segmentation type is used in this study to detect each pixel of each class object and is given a different label/color value using the R-CNN mask (regional convolutional neural network). Mask R-CNN is an algorithm that can accurately detect the target object and accurately segment the target. This paper proposes the ResNet101 structure of the original backbone network from Keras and TensorFlow. The feature maps obtained through the above backbone network will be used as the input of the Region Proposal Network (RPN), which generates region proposals with the probability of being an object for each feature map respectively. Finally, the mask is caused by the complete convolution network to obtain the area where single and overlapping groups of red blood cells are located.

A total of 5 data splitting treatments were used in this study, including 80:20, 75:25, 70:30, 65:35, and 60:40. In machine learning, data splitting is generally performed to prevent overfitting, which occurs when a machine learning model fits its training data too well and fails to fit further data correctly. We may determine the model's ability to generalize to new data sets by evaluating machine learning with varying data partitioning [34-36].

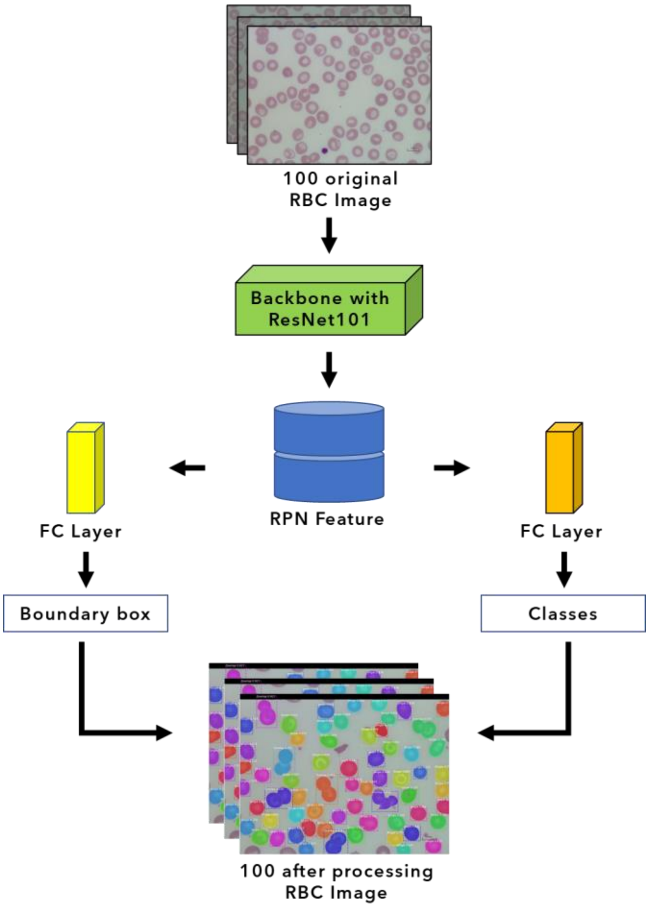


Figure 1. Overall model structure of mask R-CNN.

As the leading methodology of deep learning, convolutional neural networks (CNNs) have demonstrated extraordinary dominance in many real-world applications over most machine-learning methods. It has been established that CNN performance is mainly dependent on its designs. To attain promising performance, the structures of cutting-edge CNNs, such as GoogleNet [37], ResNet [38], and DenseNet [39], are carefully created by domain experts with extensive data and CNNs expertise. Jupyter Notebook runs all procedures in Python 3.8.8 in an Anaconda environment. The scripts utilize a Scikit-learn package with numerical Scipy and NumPy [40].

B. Evaluation of performance classification

Research prefers how many parameters are used in the performance analysis of classification algorithms [41-43]. The confusion matrix is one of them. A confusion matrix is a trendy measure used for solving classification problems. A confusion matrix is a matrix used to evaluate a classification model's performance from the number of target classes. The matrix compares the actual target values with those predicted by the machine learning model. Therefore, a confusion matrix can perfectly analyze the potential of a classifier.

Some of the commonly used parameters include precision, recall, F1-score, and acuracy [44-46]. Precision is a metric that tells us about the quality of positive predictions. Precision is calculated as the number of correct positive predictions divided by the total number of positive predictions (Equation 1). Recall tells us how well the model identifies true positives. Recall is calculated as the ratio of Positive samples correctly classified as Positive to the total number of Positive samples (Equation 2). Recall measures the ability to detect positive samples. The higher the recall, the more positive samples detected. The F1-score is the harmonic mean of precision and recall (Equation 3). The F1-score is a machine learning evaluation metric that measures the accuracy of a model. It combines the precision and recall scores of a model. A good F1-score means that you have low false positives and low false negatives, so you are correctly identifying real threats and are not disturbed by false alarms. An F1-score is considered perfect when it is 1, while the model is a total failure when it is 0. Accuracy is the degree of closeness between a measurement and its actual value (Equation 4). Accuracy tells how many times the model was correct overall.

$$\mathrm{P} = \frac{TP}{TP+FP} \tag{1}$$

$$\mathrm{R} = \frac{TP}{FN+TP} \tag{2}$$

$$F = \frac{2\times R\times P}{R+P} \tag{3}$$

$$A = \frac{TP+TN}{TP+TN+FP+FN} \tag{4}$$

Where P is precision, R is recall, F is F1-score, A is accuracy, TP is true positive, TN is true negative, FP is false positive, and FN is false negative.

In addition, a statistical test was conducted in the form of a paired *t*-test to determine the difference between the newly introduced and previous techniques [47-49]. Paired sample t-Test is a different test of two paired samples. This test aims to see if there is an average difference between two paired or related samples. Paired samples are the same subject but experience other treatments. This test model is used to analyze the research model before and after.

Also, the ANOVA test is the initial step in analyzing factors that affect a given data set [50-52]. Anova is a statistical analysis that examines the difference in means between groups. In contrast to the t-test, which can only test the mean difference between two groups, the ANOVA can test the difference between more than two groups. The group here can mean a group or type of treatment. In this study is the effect of the data splitting ratio on the accuracy of the classification model.

III. Result and Discussions

The primary objective of image segmentation is to divide an image into a zone of homogeneous representation that corresponds to the subject of interest in the picture. Image segmentation is an essential system component because it enables the actual extraction of foreground objects, which is required for following operations such as analysis and object recognition. The true success of a method for overlap-ping classification of RBCs is contingent on its capacity to correctly partition the RBC region in the observed pictures. An example of the RBC sample used in this study is presented in Figure 2.

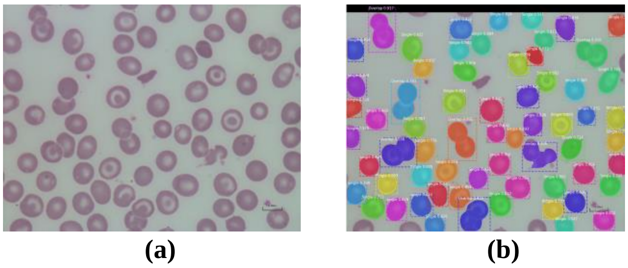
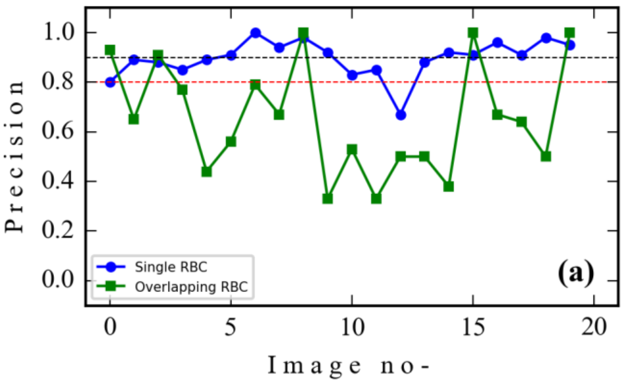


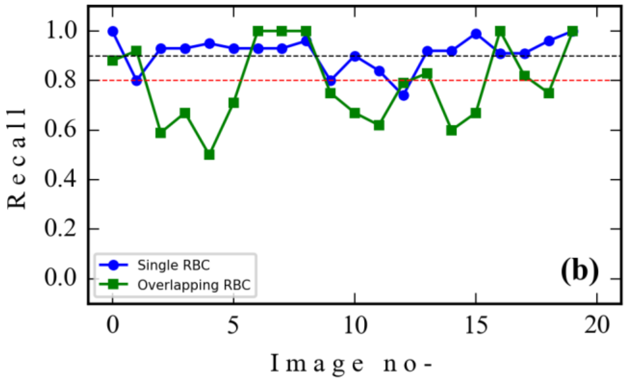
Figure 2. Example RBC image samples (a) raw image samples for testing (b) image samples results

A. Performance CNN algorithm using 80:20 data splitting

The performance of the CNN algorithm with image segmentation in classifying RBC into single and overlapping groups using a split data rate of 80:20 is presented in Figure 3. Using the confusion matrix parameters, including precision, recall, and F1-score for the single RBC class for 80:20 data splitting, will produce values of 0.90 ± 0.07, 0.91 ± 0.07, and 0.91 ± 0.05, respectively. For classification into groups, the overlapping is 0.66 ± 0.22, 0.79 ± 0.16, and 0.70 ± 0.17, respectively. Furthermore, the performance of precision, recall, and F1-score parameters is significantly different in classifying single and overlapping RBC groups at a probability of 0.05 ($p<0.05$).

Generally, a single RBC is easier to recognize by the R-CNN mask algorithm than the 20-image data tested. Only in the 9, 16, and 20 image samples did the level of precision in classifying single and overlapping groups of RBC have the same good model performance above 90%. In addition, in the 7, 8, 9, 17, and 20 image samples, the classification model has a recall performance parameter above 90%. A classification model with F1-score parameters above 90% is found in sample images 9 and 20. The confusion matrix parameter values described above show that the model produced in this study is included in the good category based on Pham [53].





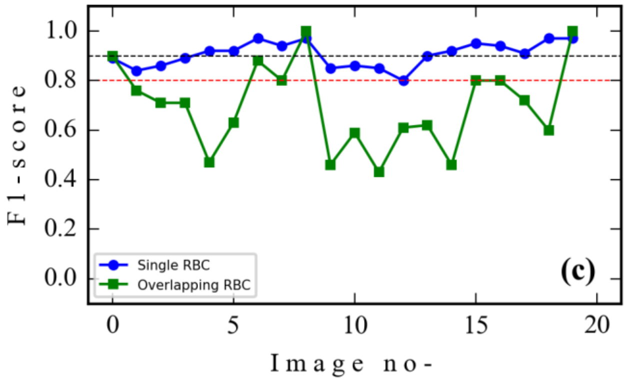


Figure 3. CNN algorithm performance with a ratio of 80:20 data splitting (a) precision, (b) recall, and (c) F1-score

B. Performance CNN algorithm using 75:25 data splitting

The performance of the CNN algorithm with a split data rate of 75:25 in classifying RBC into single and overlapping groups is presented in Figure 4. Precision, recall, and F1-score for classifying single RBC for 75:25 data splitting are 0.89 ± 0.05 , 0.94 ± 0.05, and 0.91 ± 0.04, respectively. For overlapping groups are 0.81 ± 0.15, 0.62 ± 0.21, and 0.68 ± 0.18, respectively. Comparison of performance of precision, recall, and F1-score parameters in classifying RBC into single and overlapping RBC groups showed significant differences in probability ($p<0.05$).

Testing with 25 RBC images with R-CNN showed that a single RBC is easier to classify than an overlapping RBC. Only in the 7, 8, 9, 14, and 17 image samples did the level of precision in classifying single and overlapping groups of RBC have model performance above 90%. In addition, in the 14 and 20 image samples, the classification model has a recall performance parameter above 90%. A classification model with F1-score parameters above 90% is found in sample images 14 and 20. The confusion matrix parameter values described above show that the model produced in this study is included in the good category [53].

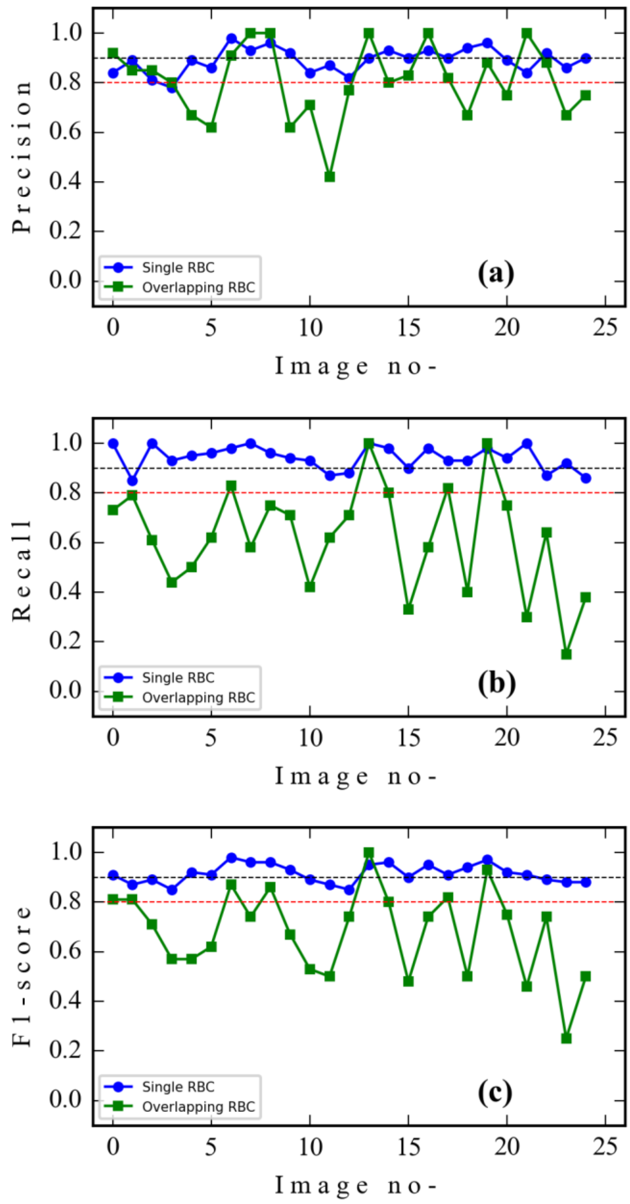


Figure 4. CNN algorithm performance with a ratio of 75:25 data splitting (a) precision, (b) recall, and (c) F1-score

C. *Performance CNN algorithm using 70:30 data splitting*

The performance of the CNN algorithm with a split data rate of 70:30 in classifying RBC into single and overlapping groups is presented in Figure 5. Precision, recall, and F1-score for classifying single RBC for 75:25 data splitting are 0.81 ± 0.09 , 0.95 ± 0.06, and 0.87 ± 0.06, respectively. For overlapping groups are 0.66 ± 0.30, 0.58 ± 0.28, and 0.60 ± 0.27, respectively. Finally, the performance of precision, recall, and F1-score parameters show a significant performance for classifying single and overlapping RBC groups for a split data rate of 70:30 at a probability of 0.05 ($p<0.05$).

Testing with 30 RBC images with R-CNN showed that a single RBC is no easier to classify than an overlapping RBC. Only in the 6, 16, 17, 23, and 30 image samples did the level of precision in classifying single and overlapping groups of RBC have model performance above 80%. In addition, the classification model that detects overlapping RBC always has a lower recall performance parameter than detecting a single RBC for all image samples. A classification model with F1-score parameters above 80% is found in sample images 2, 6, 7, 8, 9, 14, 17, 18, and 20. The confusion matrix parameter values described above show that the model produced with this splitting data is acceptable [54].

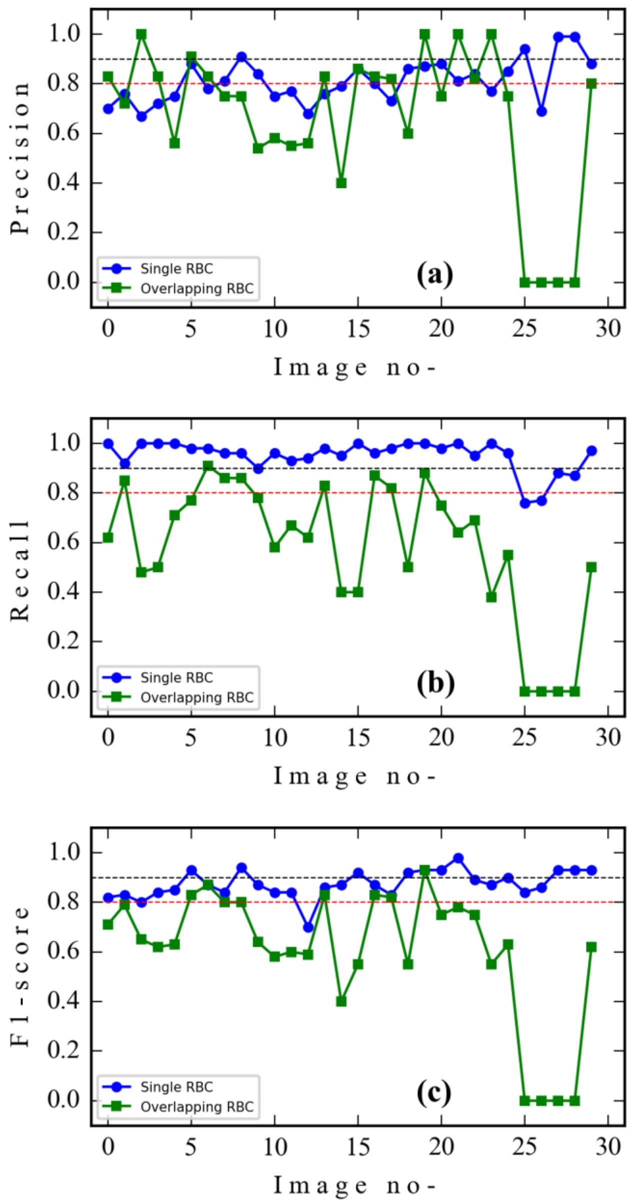


Figure 5. CNN algorithm performance with a ratio of 70:30 data splitting (a) precision, (b) recall, and (c) F1-score

D. *Performance CNN algorithm using 65:35 data splitting*

The performance of the CNN algorithm in classifying RBC into single and overlapping groups with a split data rate of 65:35 is presented in Figure 6. Precision, recall, and F1-score for classifying single RBC for 65:35 data splitting are 0.90 ± 0.06 , 0.86 ± 0.08, and 0.88 ± 0.05, respectively. For overlapping groups are 0.50 ± 0.25, 0.69 ± 0.29, and 0.52 ± 0.21, respectively. In addition, the performance of precision, recall, and F1-score parameters show a significant performance for classifying single and overlapping RBC groups for a split data rate of 65:35 at a probability of 0.05 ($p<0.05$).

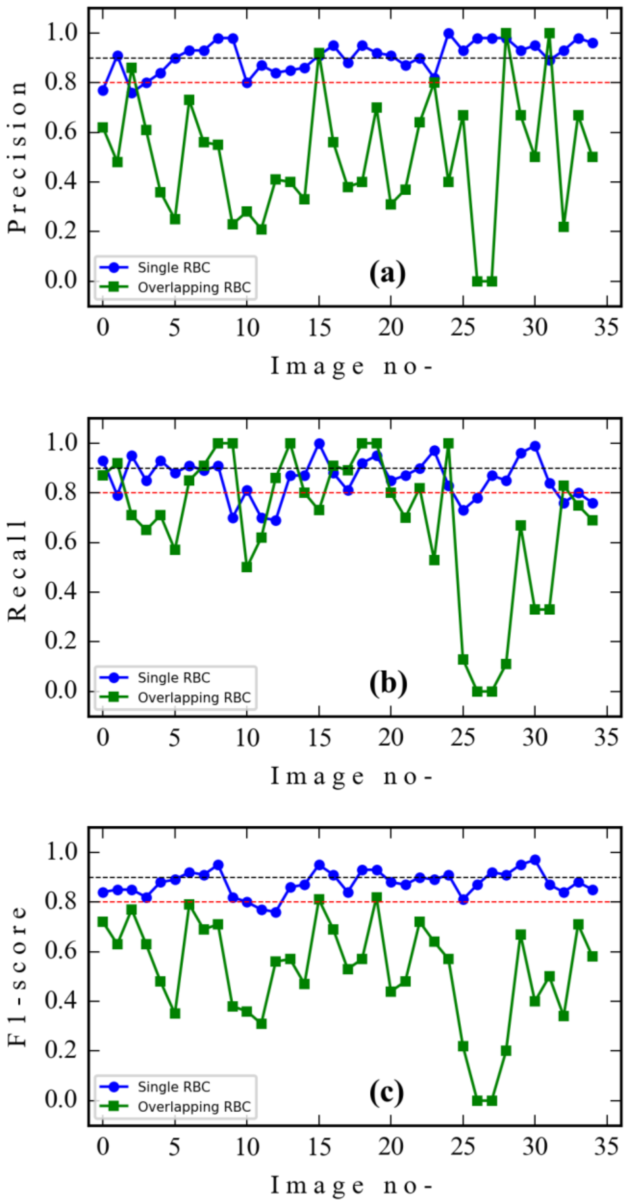


Figure 6. CNN algorithm performance with a ratio of 65:35 data splitting (a) precision, (b) recall, and (c) F1-score

Testing with 35 RBC images with R-CNN showed that a single RBC is easier to classify than an overlapping RBC. Only in the 29 and 32 image samples did the level of precision in classifying single and overlapping groups of RBC have model performance above 90%. In addition, in the 9, 19, and 20 image samples, the classification model has a recall performance parameter above 90%. A classification model with F1-score parameters that detects overlapping RBC always has a lower recall performance parameter than detecting a single RBC for all image samples. The confusion matrix parameter values described above show that the model produced in this study is included in the good category [53].

E. Performance CNN algorithm using 60:40 data splitting

The performance of the CNN algorithm with a split data rate of 60:40 in classifying RBC into single and overlapping groups is presented in Figure 7. Precision, recall, and F1-score for classifying single RBC for 65:35 data splitting are 0.90 ± 0.07 , 0.91 ± 0.07, and 0.90 ± 0.05, respectively. For overlapping groups are 0.69 ± 0.30, 0.60 ± 0.28, and 0.60 ± 0.25, respectively. Finally, the performance of precision, recall, and F1-score parameters show a significant performance for classifying single and overlapping RBC groups for a split data rate of 70:30 at a probability of 0.05 ($p<0.05$).

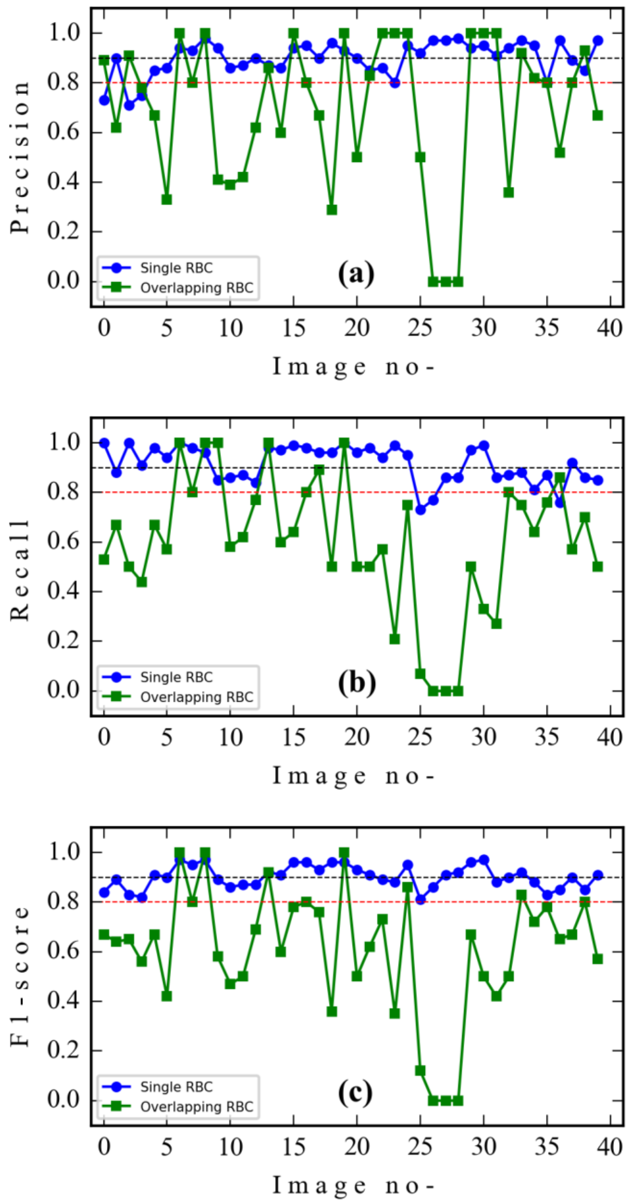


Figure 7. CNN algorithm performance with a ratio of 60:40 data splitting (a) precision, (b) recall, and (c) F1-score

Testing with 40 RBC images with R-CNN showed that a single RBC is easier to classify than an overlapping RBC. Only in the 7, 9, 16, 20, 25, 30, 31, 32, and 34 image samples did the level of precision in classifying single and overlapping groups of RBC have model performance above 90%. In addition, in the 9, 19, and 20 image samples, the classification model has a recall performance parameter above 90%. A classification model with F1-score parameters above 90% is found in sample images 6, 8, and 14. The confusion matrix parameter values described above show that the model produced in this study is included in the good category [53].

F. Overall performance of model

An overall comparison of model performance to classify RBC in single and overlapping groups is presented in Figure 8. The average accuracy of all splitting data treatments is 0.80 ± 0.03. Maximum and minimum accuracy occurs in data splitting at 75:25 and 70:30, respectively. In general, the results of the ANOVA test (Table 1) on the splitting data treatment of the model were found to have no significant effect on model performance, even at a splitting data ratio of 60:40. This shows that the single and overlapping RBC classification models developed using image segmentation are robust and unaffected by data splitting.

Source of Variation	**SS**	**df**	**MS**	**F**	**p-value**
Between Groups	0.075	4	0.0188	2.7612	0.0299
Within Groups	0.987	145	0.0068		
Total	1.062	149			

Table 1. ANOVA from the effect of data splitting on model accuracy

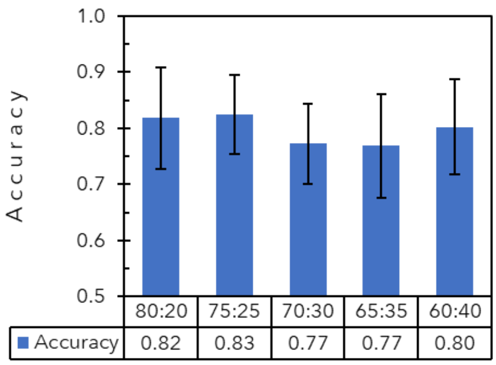


Figure 8. Comparison of the accuracy of the splitting data treatment in the single and overlapping RBC classification model

IV. Conclusion

The image segmentation process to be able to classify single and overlapping RBCs using the CNN algorithm has been studied and reported in this article. Using 100 public RBC image datasets, it is known that the CNN algorithm can classify single and overlapping RBCs with the help of Image segmentation. Model performance bias is clarified by treating data splitting starting from a ratio of 80:20 to 60:40 to get a robust model. In addition, the statistical probability test on the precision, recall, and F1-score parameters in classifying RBC into single and overlapping RBC shows significant significance. Furthermore, the accuracy resulting from the splitting data treatment was also analyzed using ANOVA and found no significant difference due to the splitting data treatment. The results of this study indicate that the Image segmentation process can be used with an accuracy rate of up to 79.7% to classify RBC into single groups and overlapping RBC using the CNN algorithm. Future work of the study is to apply the model found with RBC image data originating from several origins to strengthen the developed model.

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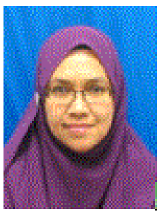

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