Effect of Data Division on Classification Performance of Overlapping Red Blood Cells using Sharpening Image Segmentation Strategy and Convolutional Neural Network

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Abstract— Red blood cell (RBC) counts and identification must be done accurately to diagnose various illnesses. Although there are drawbacks to automated image analysis methods compared to hand counting, they include differences in picture characteristics and overlapped cells. This study aimed to create a robust model that can classify overlapping RBCs by employing a region-based convolution neural network (R-CNN) with several data divisions and a sharpening picture segmentation technique. Three sharpening approaches, wand, clahe, and Otsu threshold, were incorporated into the assemble boosting model utilizing 100 RBC image datasets from imagebank.hematology.org. The integrated strategy sought to improve image segmentation quality before employing R-CNN for classification. The model's effectiveness was evaluated at many data split percentages, ranging from 80/20 to 60/40. Across many data splits, the model's average accuracy standard was 0.82 ± 0.09. It performed best when the data split was 75/25. Statistical analysis confirmed Significant performance differences between these groups, and overlapping RBC categorization caused more significant difficulties than single RBCs. The model's robustness was demonstrated by its performance, which was resistant to variations in data splits. RBCs were successfully categorized using the combined sharpening and R-CNN technique, especially single cells. Overlapping RBC categorization is still tricky, highlighting the need for more study in this field. Future advancements in biological image analysis have a promising precedent thanks to the model's stability across different data splits.

Index Terms— RBC, classification, image processing, CNN, overlapping

I. INTRODUCTION

THE identification and enumeration of red blood cells (RBCs) is a significant health indicator because it provides valuable information about a person's overall health and well-being. Red blood cells (RBC), also known as erythrocytes, carry nutrients from the respiratory system to all body parts [1-3]. An odd number of red blood cells can

indicate numerous health conditions, such as anemia, polycythemia, and iron deficiency. By counting red blood cells, healthcare professionals can accurately diagnose and treat these conditions, ensuring that a person's body receives sufficient oxygen and maintains their overall health [4-6].

Image processing is a non-destructive technique that has recently evolved from the challenging engineering field to the soft engineering field [7-11]. Using image analysis techniques, RBCs can be counted from an image. Typically, these methods involve preprocessing the image to eliminate noise and improve the visibility of the RBCs, followed by segmentation to separate the RBCs from the background. Individual RBCs can be identified and enumerated using aggregate analysis, morphological operations, or machine learning algorithms following segmentation [12, 13]. Ensuring the RBC count is accurate by verifying the results and adjusting the image analysis parameters is essential. Using automated image analysis techniques for RBCs counting can improve the process's efficiency and accuracy while decreasing the time and effort required for manual counting.

Advanced technology has been widely developed as an alternative method to identify and quantify blood cells. This technology utilizes image-processing techniques and methodologies to turn prepared blood cells into a picture and evaluate them. This approach is anticipated to have a lower cost and be more straightforward. However, unlike conventional blood cell identification and counting methods, this technique has not yet reached its maximum accuracy. Several research findings have compared performance precision with the interpretation of a hematologist [14-16].

Red blood cell counts can be calculated using various methods, including manual enumeration, automated image analysis, and flow cytometry [17-19]. Manual counting entails the examination of a blood sample under a microscope and the enumeration of RBC, a laborious process that is susceptible to discrepancies among observers. Automated image analysis employs computer algorithms to process blood sample images and automatically count red blood cells. This technique may be more objective and efficient than manual count. However, variations in shape, size, color, and other cellular structures and anomalies in the image can affect accuracy. Flow cytometry, on the other hand, employs laser-based technology to assess the physical characteristics of RBCs, such as size and granularity, and to enumerate them accurately, quickly, and automatically.

Each of these strategies possesses distinct benefits and drawbacks, and the selection of the method will be contingent upon the application's unique requirements.

The counting of RBCs using image analysis techniques lacks sufficient research. Despite advances in computer vision and machine learning, accurately identifying and counting RBCs in complex images remains challenging. This is partly due to variations in the image's shape, size, overlapping cells, color, and other cellular structures and anomalies. Furthermore, the lack of standard data sets and evaluation metrics makes it difficult to assess the performance of various algorithms and compare their results. To address these research gaps, additional effort is required to develop robust and accurate image analysis methods for the counting of RBCs and to establish standard data sets and evaluation metrics for comparison and validation. However, to the best of our knowledge, the problem of single-cell classification and cell overlap must be resolved before precisely calculating the number of cells. The algorithm for counting blood cells is perturbed and noisy when the blood cells overlap. This will considerably compromise the accuracy of the algorithm used to calculate the number of blood cells.

The categorization of RBCs might be characterized as a challenge involving the identification of patterns. It is among the most popular RBC classifiers due to its stellar reputation. Numerous studies have demonstrated the superiority of neural network classification over statistical pattern categorization. Significant classification efficiencies were 80% for typical cells and 60% for abnormal cells. Nevertheless, resolving the issue of red blood cell overlap is challenging, leading to diminished precision in forecasting the quantity of red blood cells in a picture. Hence, the primary aim of this paper was to create a model that can accurately categorize overlapping RBCs by utilizing a sharpening image segmentation technique and a region-based convolution neural network (R-CNN). Also, testing by performing some data division is analyzed to obtain a robust model.

II. MATERIALS AND METHODS

A. RBC data description

The effectiveness of the suggested technique was assessed using one hundred sets of RBC imaging data. RBC images were obtained from the website imagebank.hematology.org (American society of hematology assembled the data set). In the case of red blood cells, researchers frequently utilize this data set to evaluate the most recent techniques, methods, or algorithms.

B. Sharpening (wand), clahe and Otsu threshold

Wand is a python library that contains image manipulation functions. It can read/write images in various formats and convert images between them. The wand library is also used for image manipulation, such as image resizing and cropping, image effects, image transformations, etc. The wand's library sharpen function is used to enhance the image in the case of sharpening red blood cell images. The sharpen function on the red blood cell image wand increases the color sharpness of the edges, trying to make the

boundaries of each red blood cell object more recognizable and highlighting the boundaries between the object and the background.

An effective technique to boost color contrast is required for the sharpening process in image processing. The American society of hematology's (ASH) image bank offers numerous examples of red blood cell images in healthy and diseased states. As a result of using various image recording devices, the samples have different observable color contrast. The primary purpose of the contrast-limited adaptive histogram equalization (CLAHE) approach is to mitigate noise by finding the matrix kernels. The process involves substituting the intensity value of each pixel in the input picture with the mean of the kernel weighting values for the pixel itself and its adjacent pixels. Therefore, this method will reduce the noise in the image of the red blood cell sample and properly do the segmentation process.

The Otsu threshold method is widely used by researchers who study image processing and recognition systems to carry out automatic image development. This technique yields a solitary intensity threshold value that partitions pixels into two distinct categories: foreground and background. This enables the execution of picture-sharpening procedures [20]. Wakaf and Jalab [21] introduced the Otsu threshold approach, which involves generating a histogram of the damaged region and using the highest or lowest value as a threshold to separate faulty items in both the front and background of the picture. The findings of this investigation demonstrate that the suggested approach surpasses current approaches in identifying flaws. Consequently, the Otsu threshold method may be employed in enhancing picture sharpness based on the outcomes of this study.

C. Combinationa and assemble boosting model algorithm for RBC's image sharpening

The assemble boosting model is a novel image-sharpening strategy combining sharpen function, clahe, and Otsu threshold. Figure 1 shows the flowchart of the proposed sharpening algorithm. The sharpen function of wand implements the gaussian function, which accepts two parameters in the form of radius and sigma (variance). wand's sharpening process is carried out on all datasets, including train data, validation, and testing. This process is also the first to be carried out where the results are integrated in parallel with two algorithms, namely clahe and Otsu Thresholding.
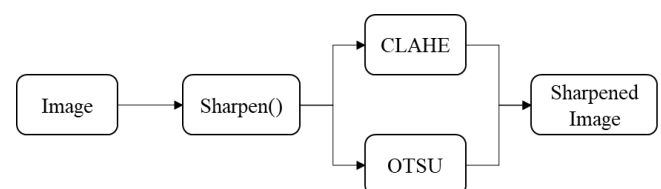

Fig. 1. Flowchart of assemble boosting model

The initial step involves utilizing the initial red blood cell picture as the input for the wand sharpen method, with the parameters radius and variance set at 20. The resultant image is then stored as a new jpg file. The output of the sharpen function of the wand is then used as input for the Otsu threshold and clahe algorithms. It combines the

assembled boosting model with R-CNN to define the limits among red blood cells and the backdrop accurately. This allows the classifier to recognize objects effectively [22]. This procedure is employed to enhance and augment the texture of a picture. This equipment enhances the clarity of the items in the image. Figure 2 depicts the combination of the assemble boosting model and the R-CNN algorithm.
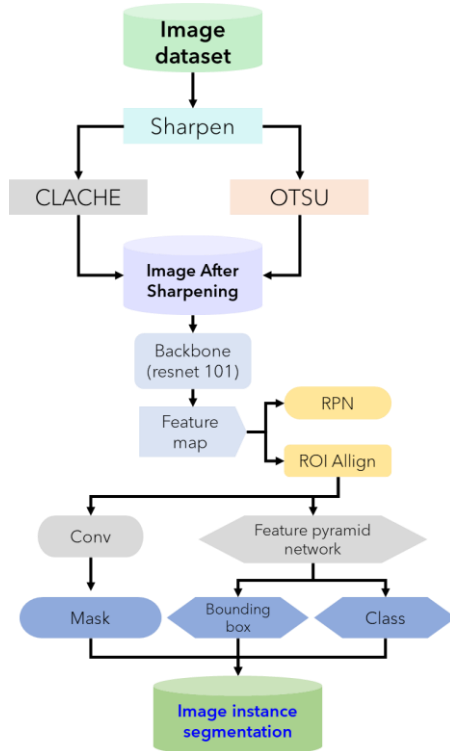

Fig. 2. Combination of the assemble boosting model and R-CNN algorithm

The CLAHE technique enhances the color contrast in a picture, making the subtle hues more pronounced [23, 24]. The CLAHE algorithm improves the precision of colors in an image by mapping the color points to a more refined set of colors. The clahe method consists of two primary processes: equalization and contrast enhancement. The equalization section divides an image in RGB format into red, green, and blue channels. Each channel retains its original image and height before being flattened into a single dimension. The image data, which consists of pixel values ranging from 0 to 255, will be categorized into 256 groups. After being standardized to a uniform size, the data from the photos will be divided into 256 groups. Subsequently, the cumulative frequency of occurrence will be computed for each group.

The Otsu threshold approach masks the backdrop to focus the model's attention on the item. The Otsu thresholding method reduces error that sometimes arises when the backdrop color resembles the object's [25]. In addition to serving as data input for clahe, the image generated by the wand sharpen algorithm also serves as data input for the Otsu threshold. The foreground (main object) and background groups are divided into two groups using the maximum variance threshold value, which is determined using the Otsu threshold algorithm. For each calculated intensity, the threshold value in pi is used. The skiimage library is used to apply the Otsu threshold; all that is left to do is carry out its functions.

To be able to distinguish between single and functioning RBCs in an image using sharpening segmentation, a robust classifier must be employed. Region-based convolutional neural networks (R-CNN) are utilized for the classification module. This algorithm detects objects in an image and generates a high-quality segmentation mask for each instance using one of the deep neural networks. Mask R-CNN is a natural extension of quicker R-CNN, but it is essential to construct the mask branch properly for optimal results.

D. Analysis of data division

The algorithm should be trained with samples representing the current class or dataset to create a high-performance model for accurate predictions on unobserved instances. If data separation is not implemented correctly, it can distribute non-representative samples for training algorithms, reducing the model's generalization and performance. In contrast, data division can mitigate the issues associated with a limited and unbalanced dataset and improve classification performance if handled carefully [26-28]. Therefore, this study treats 5 data divisions (calibration/testing), including 80/20, 75/25, 70/30, 65/35, and 60/40.

E. Evaluation of model classification

Research favors using many parameters in the performance analysis of classification algorithms. The matrix of confusion is one of them. A confusion matrix is a popular method of solving classification issues. A matrix of confusion is a grid utilized to assess the efficacy of a classification model by considering the count of target categories. The grid compares the absolute objective values with the values forecasted by the machine learning algorithm. Hence, a confusion matrix may accurately evaluate the efficacy of a classifier [29, 30].

Precision, recall, and the parameters that are F1-score are a few of the frequently used. Precision is a metric that indicates the accuracy of positive predictions. Precision is calculated by dividing the count of correct positive forecasts by the overall count of optimistic predictions. Recall refers to the precision with which an equation correctly recognizes true positives. A recall is determined by dividing the number of correctly recognized positive samples by the overall amount of positive samples. Recall measures the capacity to identify positive samples accurately. As the number of recalled items rises, a more significant number of positive samples are determined. The F1-score is a metric that quantifies the harmonic mean of accuracy and recall. The F1-score is a machine learning that evaluates a model. Integrates a model's precision and recall scores. A strong F1-score indicates that you have few false positives and few false negatives, allowing you to correctly identify genuine threats without being disturbed by false alarms. An F1-score of 1 is considered flawless, while a score of 0 indicates complete failure.

In addition, a paired t-test was conducted to determine the difference between the newly implemented and previous techniques. The paired sample t-test is an alternative test for comparing two paired samples. This test determines whether

two paired or related samples differ on average [31, 32]. In paired samples, the same subject is subjected to different treatments. Before and after analyzing the research model, this test model is utilized.

III. RESULTS AND DISCUSSIONS

A. Data division 80/20

The classification model's performance is much enhanced by combining the contrast limited adaptive histogram equalization (CLAHE), Otsu threshold, and sharpened approaches. As seen in Fig. 3, these techniques combined improve the outcomes in distinguishing RBC into distinct and overlapping groups. For this investigation, an 80/20 data division rate was used. The maximum accuracy attained is 0.81 ± 0.09. The assemble boosting model was also used to examine the single RBC class utilizing the confusion matrix's characteristics, including accuracy, recall, and F1-score. In addition to the three methods mentioned above (the clahe, Otsu threshold, and wand functioning together), the data division produced F1-score, precision, and recall values of 0.90 ± 0.05, 0.96 ± 0.05, and 0.86 ± 0.07, respectively.
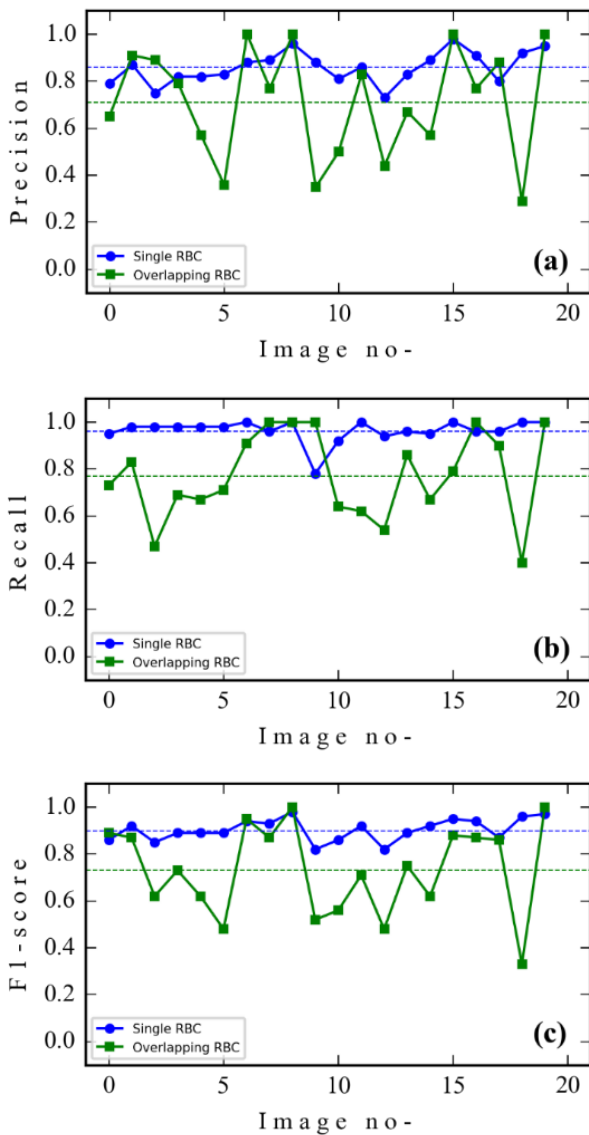

Fig. 3. Classification performance model of overlapping RBCs with data division 80/20 (a) precision, (b) recall, and (c) F1-score

The focus of the investigation that followed was grouping RBCs into overlapping categories. Despite being more complicated, this field has a lot of interest. For this category, the recorded values for accuracy, recall, and F1-score were 0.71 ± 0.24, 0.77 ± 0.19, and 0.73 ± 0.19, in that order. These numbers highlight the underlying complexity involved with overlapping groups, even if they are lower than those for single RBCs. Notably, the overlapping categorization was affected by the complex spatial configurations and the physical closeness of the RBCs.

Moreover, a crucial finding of the study was the differential in the performance metrics between individual and overlapping categories when categorizing RBCs. This was demonstrated by statistical analysis, which found a significant probability value of 0.05 ($p<0.05$) [33]. This result highlights how challenging it is to differentiate between overlapping and isolated RBCs. Of the factors that have been assessed, it is clear that the classification problems related to single RBCs are significantly more straightforward than those associated with overlapping groups. This finding emphasizes even more how important it is to conduct research and optimization in this area to improve classification accuracy.

B. Data division 75/25

The section focuses on the performance of the assemble boosting model, a novel machine learning method with a data divide of 75/25. This data division is essential to train the model and confirm its correctness. Three different ways of sharpening were included in the model to improve the quality of the images: wand, clahe, and Otsu threshold. Combined, these techniques significantly increase the ability to see and distinguish red blood cells (RBCs) in microscopic pictures. Fig. 4 presents the results of this synergistic strategy in detail. A critical consequence of this data split is the accuracy of the model. The information demonstrates that the single RBC detection problem produced better results than the overlapping RBC detection problem. A considerable maximum accuracy of 0.88 ± 0.07 serves as a measure for this.

Several essential indicators become apparent when we examine the 75/25 data partition in more detail. Precision, recall, and the F1 score stand out in a single RBC classification as critical performance metrics. The robustness of the model was demonstrated by the reported values of 0.92 ± 0.07, 0.96 ± 0.04, and 0.94 ± 0.04 for these measures. However, when overlapping RBC groups were classified, the results naturally decreased significantly, measuring 0.82 ± 0.16, 0.86 ± 0.13, and 0.83 ± 0.12. This kind of comparison clearly illustrates the additional difficulties caused by overlapping RBC detection.

Moreover, statistical analysis confirms the considerable discrepancy in performance between these two groups. The results indicate that the single RBC problem performs significantly better than the overlapping RBC problem, with a significant probability of 0.05 ($p<0.05$). This disparity highlights possible areas of emphasis for further improvement and streamlining in RBC categorization.
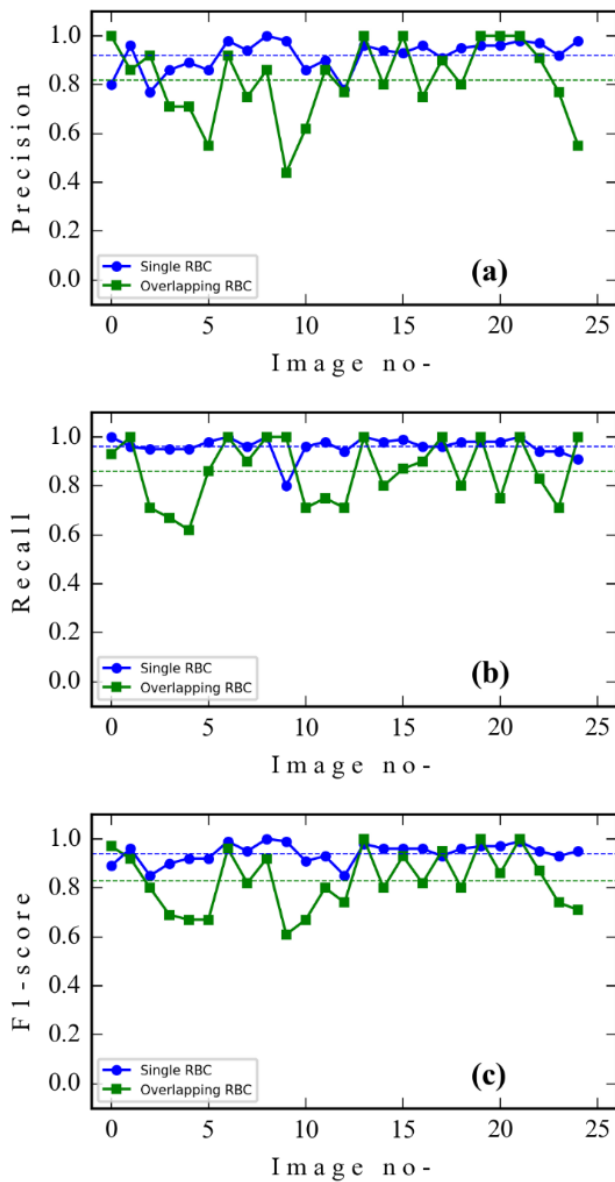
Fig. 4. Classification performance model of overlapping RBCs with data division 75/25 (a) precision, (b) recall, and (c) F1-score

C. Data division 70/30

In medical image processing, sophisticated machine learning methods have completely changed the field of microscopic analysis. With a data partition of 70/30, the assemble boosting model—a cutting-edge classification mechanism—has been carefully assessed. This partitioning technique ensures a balanced split for the model's testing and training. Three sharpening methods—wand, clahe, and Otsu threshold—are used in this procedure to improve the clarity of the images. Its combined application has dramatically enhanced the model's capabilities. A thorough graphic depiction of these results can be seen in Fig. 5, demonstrating how well various sharpening techniques work together to differentiate between red blood cells (RBCs) and whether they overlap with other cells or stand-alone.

Looking more closely at the analytical findings, it becomes clear that the preprocessing approach with the 70/30 data split had a significant impact. Interestingly, at a significance level of 0.05 ($p>0.05$), this method had no

discernible effect on recall, accuracy, and F1-score performance measures. These results validate the model's ability to classify RBCs into single or overlapping groups with an astounding maximum accuracy of 0.82 ± 0.09. According to specific criteria, the accuracy, recall, and F1-score values for the single RBC categorization were 0.93 ± 0.06, 0.89 ± 0.09, and 0.91 ± 0.05. Conversely, the measures were marginally lower at 0.68 ± 0.20, 0.80 ± 0.18, and 0.71 ± 0.16 when addressing overlapping RBC groups. The comparison analysis confirms that single RBC detection performs better than its overlapping equivalent, with a probability value 0.05 ($p<0.05$).
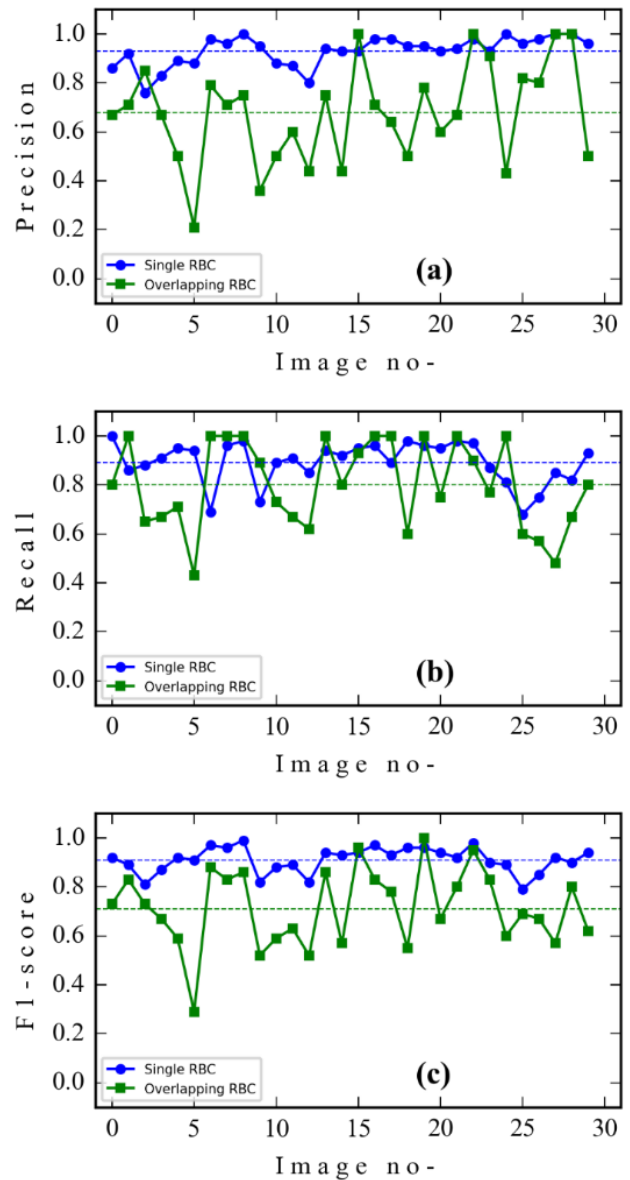

Fig. 5. Classification performance model of overlapping RBCs with data division 70/30 (a) precision, (b) recall and (c) F1-score

D. Data division 65/35

The categorization of red blood cells (RBCs) is essential for many medical research and diagnostic projects. A robust computational model is needed to recognize and classify individual RBCs in this process—significantly when differentiating between single and overlapping cells. Assemble boosting is a promising model incorporating

ensemble techniques to increase predicting accuracy and decrease overfitting. This approach is crucial to dividing the data into training and testing sets. A 65/35 split was used in this study, which means that 65% of the data was set aside for training and the remaining 35% for validation and testing. The precise outcomes of this specific data split approach are shown in Fig. 6. The graphical depiction demonstrates the effectiveness of the assemble boosting model and provides evidence of the powerful union of medical imaging and machine learning.
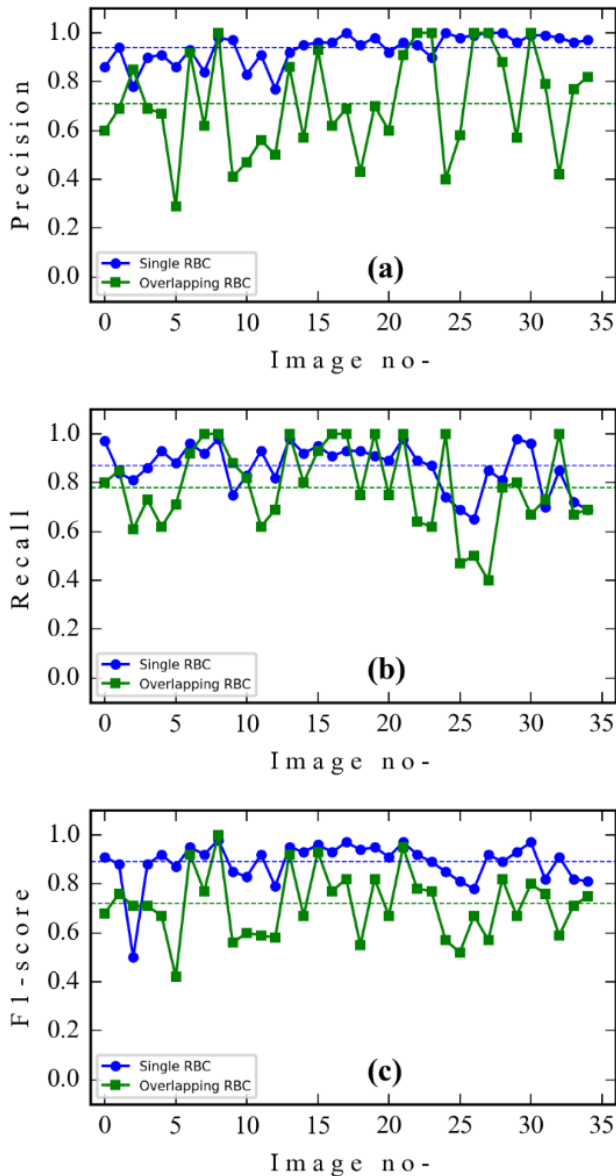

Fig. 6. Classification performance model of overlapping RBCs with data division 65/35 (a) precision, (b) recall, and (c) F1-score

Examining the numerical details reveals that the model's effectiveness differs according to the kind of RBC classification. The model's most excellent accuracy for a single RBC categorization is an astounding 0.80 ± 0.10. Analyzing this further, we find that the precision, recall, and F1-score metrics are, respectively, 0.94 ± 0.06, 0.87 ± 0.10, and 0.89 ± 0.09. These numbers show that the model performs exceptionally well in recognizing isolated RBCs. On the other hand, there is a noticeable decrease in performance when the model is asked to categorize

overlapping RBC groups; the metrics for accuracy, recall, and F1-score, respectively, read 0.71 ± 0.21, 0.78 ± 0.17 and 0.72 ± 0.13. This disparate performance shows the fundamental difficulties in differentiating overlapping cells. Nonetheless, the results unquestionably imply that the single RBC categorization works better than its overlapping counterpart compared to the statistical significance level of 0.05 ($p<0.05$). These findings have broad ramifications that might affect future RBC categorization research approaches and techniques.

E. Data division 60/40

In the rapidly evolving medical diagnostics and research field, accurately classifying red blood cells (RBCs) has become a critical endeavor. This categorization provides insights into more considerable physiological consequences in addition to helping to understand hematological disorders. Computational models are continuously improved as part of this research continuum to improve their accuracy in distinguishing between single and overlapping RBCs. An essential component of effectively implementing these models is the data division technique used for training and validation. We explore the subtleties of a 60/40 data split, in which 40 percent of the dataset is used for verification and 60 percent for training. In Fig. 7, the empirical results obtained using this method are methodically displayed. In addition to providing a clear grasp of the model's effectiveness in RBC categorization, this visual aid also compares the model's performance to alternative data division methodologies currently being discussed in scientific circles.

Some noteworthy findings emerge from carefully analyzing the 60/40 data split results. The most important is the model's performance difference according to the kind of RBC being categorized. In particular, individual RBCs had an excellent maximum accuracy of 0.80 ± 0.10. Examining this classification type in more detail reveals that the F1-score, precision, and recall metrics are, respectively, 0.90 ± 0.06, 0.88 ± 0.09, and 0.93 ± 0.07. Strong performance indicators show how well the algorithm can identify and categorize isolated RBCs. By contrast, the model's performance decreased when overlapping RBC groups were present. The metrics for accuracy, recall, and F1-score were 0.63 ± 0.21, 0.78 ± 0.20, and 0.67 ± 0.15, respectively, for this category. This significant disparity highlights the intrinsic difficulties in identifying overlapping cells. A statistical analysis, calibrated against a significance level of 0.05 ($p<0.05$), clearly shows that the single RBC categorization is better than the overlapped category. These findings have a great deal of potential for improving computational models in the future and the overall approach to RBC categorization.

F. Comperasion performance of the model

Accurately categorizing Red Blood Cells (RBCs) into distinct categories of single or overlapping groups has always been a topic of interest and research in the quickly developing field of biomedical informatics. This categorization has significant consequences for comprehending different hematological diseases; it goes beyond academic curiosity. Our study aimed to use cutting-edge computational modeling methods to tackle this

problem. It is essential to understand the depth of this research, which analyzes the model's performance across a wide range of data split ratios, as we show our results in Figure 8. After thorough analysis, an average accuracy standard of 0.82 ± 0.09 is found. It is instructive to see the highs and lows of the model's performance; the maximum was reached at a 75:25 data split, while the lowest points were recorded at 65:35 and 60:40 data splits.
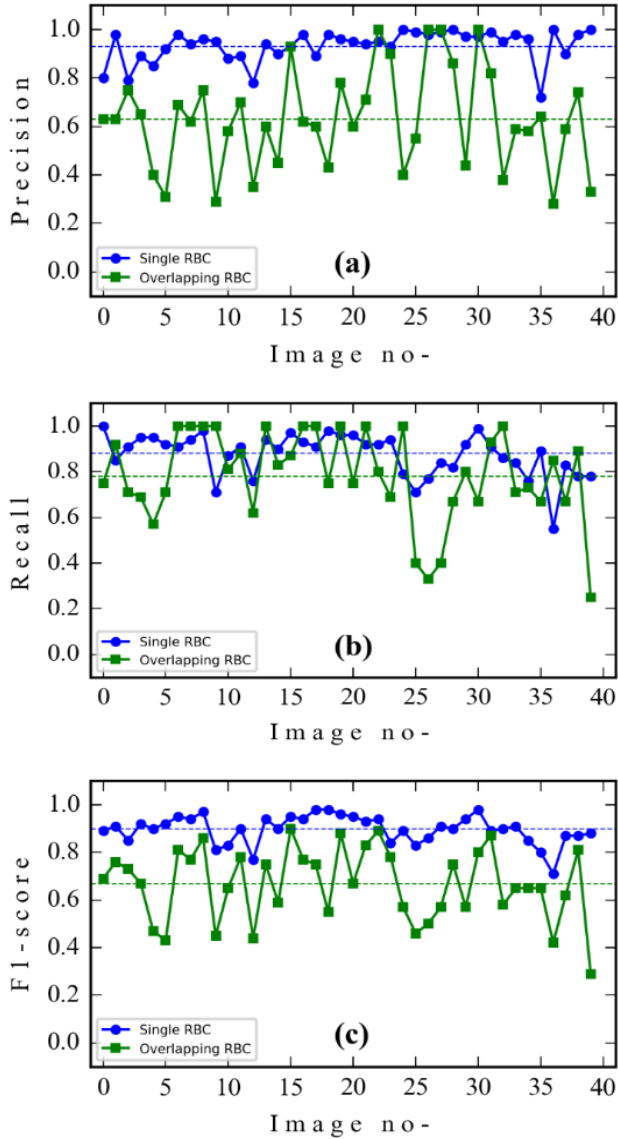

Fig. 7. Classification performance model of overlapping RBCs with data division 60/40 (a) precision, (b) recall, and (c) F1-score

Although these performance indicators provide essential information, determining the model's robustness to changes in data splitting is just as important, if not more so. Examining the ANOVA test findings in detail (see TABLE I for references) shows an interesting story. In contrast to the expected differences in split ratios, the models demonstrated excellent performance consistency. The model remarkably maintained its stability at a divided ratio as lopsided as 60:40. This reflects the strength of the classification model created with the assemble boosting methodology.

The three sharpening procedures included in this model— wand, clahe, and Otsu threshold—further enhance its effectiveness. Their combined effect appears to be the

keystone that prevents this model from being affected by changes in the data split. This result emphasizes algorithmic robustness's significance in biological classifications, bodes well for the current study, and establishes a precedent for future attempts.
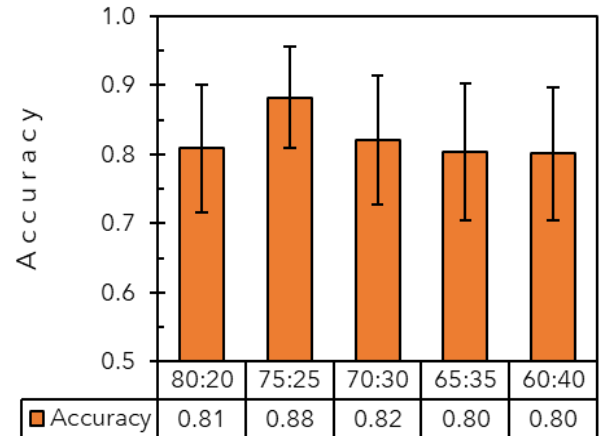

Fig. 8. Comperasion performance every data splitting

TABLE I
ANOVA FROM THE EFFECT OF DATA SPLITTING ON MODEL ACCURACY

Source of variation	SS	df	MS	F	*p*-value
Among different groupings	0.124	4	0.031	3.605	0.008
Amongst groupings	1.242	145	0.009		
Total	1.366	149			

IV. CONCLUSIONS

A fundamental aspect of biomedical informatics, categorizing Red Blood Cells (RBCs) into discrete categories of single or overlapping groups has ramifications beyond scholarly research. It may influence our comprehension of various hematological illnesses. This study demonstrated the effectiveness of advanced computational modeling in tackling this problem, especially when the assemble boosting approach is used in conjunction with three different sharpening techniques: wand, clahe, and Otsu threshold. An accuracy benchmark with an average of 0.82 ± 0.09 was determined by carefully investigating several data split ratios. Surprisingly, the model demonstrated its peak performance at a 75:25 data split but was still resilient at even more skewed divisions, such as 60:40.

A critical lesson from this study is how much more difficult it is to classify overlapping RBCs than isolated ones. Even with cutting-edge methods, there is still a noticeable difference in performance between these two classifications. This discovery was confirmed by statistical analysis, which also highlighted the better performance in the single RBC group. Future research attempts are presented with both a problem and an opportunity because of the inherent difficulty of identifying overlapping cells. However, the model's resilience to variations in data splits and its sharpening techniques provide a solid basis for future improvements in this area of research. The study established a robust framework that emphasizes the significance of

algorithmic resilience in biological classifications and provides a standard for further investigation.

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