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Classification of Overlapping Red Blood Cells in Microscopic Blood Smear Images Using Deep Learning

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Abstract

Automated analysis of microscopic blood smear images plays a crucial role in modern hematological diagnosis. While existing computer vision and deep learning techniques have demonstrated strong performance in detecting and counting isolated blood cells, the classification of overlapping red blood cells (RBCs) remains a challenging problem due to ambiguous boundaries and dense cellular arrangements. Traditional image processing methods often fail under such conditions, leading to inaccurate cell counts and potential diagnostic errors. In this work, a deep learning–based framework for the classification of overlapping and non-overlapping red blood cells is presented. A real microscopic dataset derived from the publicly available Blood Cell Count Dataset (BCCD) is utilized. Red blood cell regions are first localized using a pretrained YOLO-based object detector, followed by a lightweight convolutional neural network for binary classification of overlapping and single RBCs. Weak supervision based on morphological area estimation is employed to generate overlap labels. Experimental evaluation on a dataset of 38 RBC samples demonstrates an overall classification accuracy of up to 80%, with strong recall for single RBCs and moderate performance for overlapping cases. The results highlight both the effectiveness and the inherent challenges of overlapping RBC classification in small and weakly supervised datasets, providing a foundation for future improvements using larger datasets and pixel-level annotations.

1. Introduction

The Complete Blood Count (CBC) test is one of the most frequently performed diagnostic procedures in clinical practice, providing essential information about a patient's health status. Red blood cells (RBCs), which constitute the largest proportion of blood components, are responsible for oxygen transport throughout the human body. Abnormalities in RBC count, shape, or distribution are key indicators of hematological disorders such as anemia, leukemia, and sickle cell disease [1], [3].

Traditionally, blood cell analysis is performed manually by trained pathologists using microscopes and hemocytometers. Although manual examination is considered reliable, it is labor-intensive, time-consuming, and prone to human error and inter-observer variability [1], [5]. To address these limitations, automated blood cell analysis systems based on digital image processing and machine learning have been widely studied.

Microscopic View of a Blood Smear Sample. This figure illustrates a typical blood smear slide and the various cellular components, including red blood cells (RBCs), leukocytes (Neutrophils, Lymphocytes, etc.), and platelets. It highlights the

baseline visual complexity involved in manual hematological examinations.

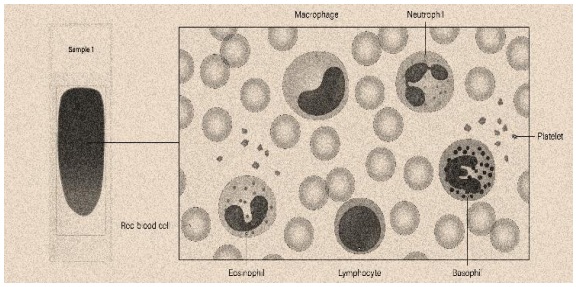

Figure 1: Representation of microscopic view of blood Smear Sample

Early approaches relied heavily on classical image processing techniques such as thresholding, edge detection, morphological operations, and Hough Transform-based circle detection [3], [19]. While these methods perform reasonably well for isolated and well-separated cells, they often fail in the presence of dense cellular regions where red blood cells overlap or touch each other. Overlapping RBCs are common in real blood smear images and pose a major challenge for segmentation-based pipelines.

Recent advances in deep learning, particularly convolutional neural networks (CNNs), have significantly improved performance in medical image analysis tasks. Object detection frameworks such as Faster R-CNN and YOLO treat cell detection as a regression or region proposal problem, enabling robust localization even in cluttered scenes [4], [12], [14]. Several studies have applied these models to blood cell detection and counting with promising results [5], [10], [11]. However, most existing works focus primarily on detection and counting, with limited emphasis on explicitly classifying overlapping RBCs as a separate category.

The classification of overlapping red blood cells is a critical yet under-explored problem. Overlapping cells can lead to under-counting, misclassification, and inaccurate clinical interpretation if not handled properly. This work aims to address this gap by proposing a practical deep learning–based framework for classifying overlapping and non-overlapping RBCs using real microscopic data and computationally efficient models.

2. Problem Statement

Despite significant progress in automated blood cell analysis, accurate classification of overlapping red blood cells remains a challenging task. Most traditional segmentation-based methods assume that RBCs are isolated and approximately circular, which is rarely the case in real blood smear images. When cells overlap, their boundaries become ambiguous, leading to merged regions or incorrect segmentation [3], [19].

Although modern deep learning-based object detectors can localize blood cells in dense images, they are generally trained to detect individual instances and do not explicitly differentiate between single and overlapping RBCs [5], [10], [11]. As a result, overlapping cells are often treated as single instances, causing errors in downstream classification and counting tasks.

Furthermore, publicly available datasets rarely provide explicit annotations for overlapping RBCs, making supervised learning difficult. The limited dataset size and class imbalance further complicate model training and evaluation. Therefore, there is a need for a robust and computationally efficient approach that can classify overlapping and non-overlapping red blood cells using weak supervision and real microscopic data.

3. Objectives of the Work

The main objectives of this research work are as follows:

1. **To study existing image processing and deep learning techniques** for blood cell detection, counting, and classification, with emphasis on their limitations in handling overlapping red blood cells [1], [3], [5].
2. **To construct a real RBC dataset** from microscopic blood smear images using a pretrained object detection model and weakly supervised labeling strategies.
3. **To develop a lightweight convolutional neural network model** for binary classification of overlapping and non-overlapping red blood cells.
4. **To evaluate the proposed framework** using standard performance metrics such as accuracy, precision, recall, and F1-score.
5. **To analyze the impact of dataset size, class imbalance, and weak supervision** on classification performance and identify key challenges for future research.

4. Literature Survey

Automated analysis of blood smear images has been an active research area for several decades due to its importance in clinical diagnosis and the limitations of manual examination. Existing approaches for blood cell detection, counting, and classification can be broadly categorized into **traditional image processing methods**, **machine learning-based approaches**, and

deep learning-based detection and classification frameworks.

A. Traditional Image Processing Approaches

Early research on blood cell analysis primarily relied on classical image processing techniques. These methods focused on exploiting the geometric and color properties of blood cells, particularly the near-circular shape of red blood cells.

Guan and Yan [19] proposed a blood cell image segmentation technique based on the Circular Hough Transform combined with fuzzy curve tracing. Their approach effectively detected circular boundaries of RBCs under controlled conditions. However, it assumed well-separated cells and struggled significantly in cases involving overlapping or irregularly shaped RBCs.

Varun and Priya [3], [9] presented a digital image processing-based blood cell counting system that employed plane extraction, edge detection, morphological operations, and Circular Hough Transform. While their system achieved reasonable accuracy for RBC and WBC counting, the authors acknowledged that overlapping cells posed a major limitation, often resulting in incorrect counts.

Meimban et al. [1] developed a Python OpenCV-based system using blob detection and color filtering to count RBCs and WBCs. Although the system achieved high accuracy for distinct cells, its performance degraded in dense smear images where RBCs overlapped, highlighting the inherent limitations of blob-based methods.

Overall, traditional image processing approaches are computationally efficient and interpretable but lack robustness when handling overlapping cells, uneven illumination, staining variations, and morphological abnormalities.

B. Machine Learning-Based Approaches

To overcome the rigidity of rule-based methods, researchers introduced machine learning techniques that relied on handcrafted feature extraction followed by classification.

Alam and Islam [5], [7] proposed a machine learning-based framework using the YOLO object detection algorithm for automatic identification and counting of RBCs, WBCs, and platelets. Their work demonstrated that learning-based models outperform traditional image processing techniques, especially in complex backgrounds. However, their primary focus was detection and counting rather than explicit classification of overlapping RBCs.

Wu et al. [6] applied radiomics-based feature extraction combined with deep learning classifiers for white blood cell image classification. While their study focused on WBCs, it demonstrated the effectiveness of combining handcrafted features with learning-based classifiers in hematological image analysis.

Mohamed et al. [8] explored automated detection of cancer-related white blood cell abnormalities using machine learning classifiers. Their work emphasized the diagnostic importance of accurate cell classification but did not address overlapping RBC scenarios.

Machine learning-based methods improved generalization compared to classical techniques but still relied heavily on feature engineering and were sensitive to overlapping and clustered cell structures.

C. Deep Learning-Based Detection and Classification

Recent advances in deep learning, particularly convolutional neural networks (CNNs), have significantly transformed medical image analysis by enabling end-to-end learning directly from raw image data.

Ren et al. [14] introduced Faster R-CNN, which combined region proposal networks with deep convolutional features for object detection. the number of foreground pixels determin

Cheng et al. [4] proposed an improved Faster R-CNN model for white blood cell detection in blood smear images. Their method enhanced the region proposal network to better detect small and densely packed cells, including partially overlapping cells. However, their work focused primarily on WBC detection rather than RBC overlap classification.

Redmon and Farhadi [12] introduced the YOLO framework, which treats object detection as a single regression problem, enabling real-time detection. Due to its speed and simplicity, YOLO has been widely adopted in blood cell detection tasks.

Guo and Zhang [11] further improved YOLOv5 for blood cell detection by integrating Squeeze-and-Excitation (SE) attention mechanisms and advanced bounding box regression losses. Their approach demonstrated improved performance in detecting small and overlapping cells, highlighting the importance of attention mechanisms in dense cellular environments.

Li et al. [10], [13] proposed a multi-label detection and classification framework for red blood cells using deep learning. Their approach explicitly addressed overlapping RBCs by treating them as multi-instance regions rather than attempting strict segmentation. This work is one of the most relevant studies addressing overlapping RBC classification, although it

requires extensive annotations and computational resources.

D. Deep Learning Architectures and Loss Functions

Several studies have focused on improving CNN architectures and loss functions to enhance performance in dense object detection scenarios. He et al. [15] introduced deep residual learning (ResNet), which enabled training of deeper networks and improved feature representation. Residual architectures are widely used as backbones in blood cell detection models.

Lin et al. [16] proposed Focal Loss to address class imbalance in dense object detection tasks. This loss function reduces the impact of easy negatives and is particularly relevant for blood cell datasets where overlapping cells are underrepresented.

Hu et al. [17] introduced Squeeze-and-Excitation networks, which adaptively recalibrate channel-wise feature responses. SE blocks have been shown to improve performance in medical image analysis, especially for small and overlapping objects.

Zheng et al. [18] proposed Distance-IoU (DIoU) and Efficient-IoU (EIoU) losses to improve bounding box regression accuracy and convergence speed in object detection models. These losses are particularly effective in crowded scenes where precise localization of overlapping objects is required.

Ronneberger et al. [20] proposed the U-Net architecture for biomedical image segmentation. While U-Net excels in pixel-level segmentation tasks, its performance degrades in heavily overlapping regions without extensive annotations.

E. Research Gap

From the reviewed literature, it is evident that:

- Traditional image processing methods fail to handle overlapping RBCs effectively.
- Machine learning approaches improve robustness but remain limited by handcrafted features.
- Deep learning-based object detectors achieve high detection accuracy but often focus on counting rather than explicit overlap classification.
- Explicit classification of overlapping RBCs remains under-explored, particularly under weak supervision and limited dataset conditions.

This motivates the need for a practical and computationally efficient framework that can classify overlapping and non-overlapping red blood cells using real microscopic data, without relying on extensive manual annotations.

5. Proposed Methodology

The proposed methodology aims to classify overlapping and non-overlapping red blood cells (RBCs) from microscopic blood smear images using a deep learning–based framework. The overall pipeline consists of **four main stages**: dataset preparation, RBC localization, overlap classification, and performance evaluation. Two models are implemented and analyzed: a **base model** and an **improved model**, enabling comparative performance assessment.

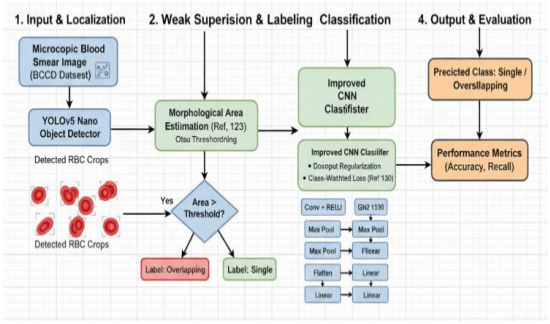

Figure 2: Architecture Flow

Proposed System Architecture for Overlapping RBC Classification: The diagram outlines the end-to-end pipeline:

1. Input and Localization using YOLOv5,
2. Weak Supervision via morphological area estimation for automated labeling,
3. Classification using an improved CNN with class-weighted loss, and
4. Final Output evaluation based on performance metrics.

A. Dataset Preparation

Microscopic blood smear images are obtained from the publicly available Blood Cell Count Dataset (BCCD). Since explicit annotations for overlapping RBCs are not provided, a weakly supervised labeling strategy is adopted. A subset of images is processed to extract red blood cell regions, resulting in dataset sizes of up to **38 RBC samples**, with a balanced distribution of overlapping and non-overlapping cells.

To address limited data availability, preprocessing steps such as resizing, normalization, and noise-based data augmentation are applied. This ensures improved generalization while maintaining computational efficiency.

B. Red Blood Cell Localization Using YOLO

A pretrained YOLOv5 nano object detection model is employed to localize red blood cells in

microscopic images. YOLO treats object detection as a single-stage regression problem, enabling fast and robust detection in dense blood smear images. The lightweight YOLOv5 nano architecture is selected to ensure rapid inference and suitability for low-resource environments [11], [12].

Detected bounding boxes are cropped and resized to a fixed resolution of 64×64 pixels for subsequent classification.

C. Weakly Supervised Overlap Labeling

Since manual overlap annotations are unavailable, RBC regions are labeled using a morphological area-based heuristic. After converting cropped RBC images to grayscale, Otsu thresholding is applied to generate binary masks. The number of foreground pixels is then computed to estimate the effective cell area. Crops exceeding a predefined area threshold are labeled as **overlapping RBCs**, while smaller regions are labeled as **single RBCs**. This weak supervision strategy enables overlap classification without requiring pixel-level annotations.

Detailed Logic for Weakly Supervised RBC Labeling. This framework shows the localized RBC crops being analyzed based on an area threshold. If the area exceeds a predefined value, the crop is labeled as 'Overlapping'; otherwise, it is classified as 'Single'. This automated labeling provides the ground truth for training the classifier.

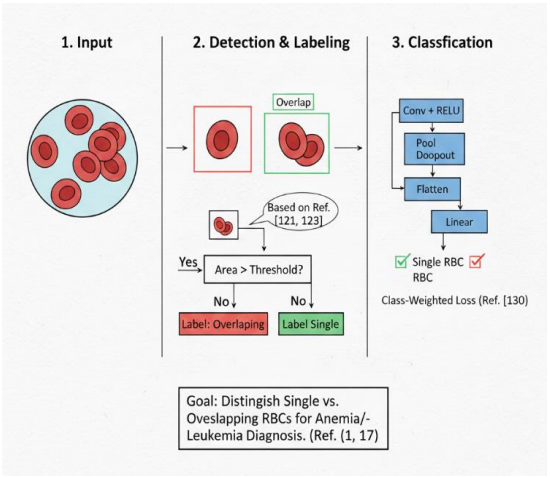

Figure 3: RBC overlapping classification

Morphological Area Estimation and Label Assignment

The morphological area estimation is derived from the pixel count of the binary mask obtained using Otsu's thresholding method. For a cropped red blood cell (RBC) region of height $H$and width W, the effective area $A$is computed as:

$$A = \sum_{i=1}^{H} \sum_{j=1}^{W} B(i,j)$$

where $B(i,j) \in \{0,1\}$represents the binary value of the pixel at spatial coordinates (i, j)after thresholding.

To distinguish between single and overlapping RBCs, the computed area is compared against a predefined threshold T. Crops satisfying:

$$A > T$$

are classified as overlapping cells and assigned the label $L = 1$, while those with:

$$A \leq T$$

are categorized as single cells and assigned the label $L = 0$.

D. Overlapping RBC Classification Using CNN

A lightweight convolutional neural network (CNN) is designed for binary classification of overlapping and non-overlapping RBCs. The **base model** consists of two convolutional layers followed by max-pooling and fully connected layers. This model serves as a baseline to evaluate the feasibility of overlap classification using limited data.

To improve performance, an **enhanced CNN model** is introduced with dropout layers for regularization and noise-based data augmentation during training. Additionally, class-weighted cross-entropy loss is employed to mitigate the impact of class imbalance between overlapping and single RBCs. These enhancements enable improved recall and F1-score for minority classes.

Overlapping RBC Classification

To mitigate the impact of class imbalance between single and overlapping red blood cells (RBCs), a class-weighted cross-entropy loss function is employed during model training. This approach ensures that underrepresented classes contribute proportionally to the optimization process.

The loss function is defined as:

$$L = -\sum_{c=1}^{2} w_c \; y_c \log(\hat{y}_c)$$

where:

- $c$denotes the class index ($c = 1$ for single RBCs and $c = 2$for overlapping RBCs),
- $w_c$represents the weight assigned to class $c$to balance its contribution to the loss,
- $y_c \in \{0,1\}$is the ground-truth label, and

- $\hat{y}_c$ is the predicted probability for class c.

By assigning higher weights to the minority class, the proposed loss formulation effectively reduces classification bias and improves recall for overlapping RBCs, resulting in more balanced and robust model performance.

E. Model Training and Evaluation

The dataset is split into training and testing sets using a hold-out validation strategy. Models are trained using the Adam optimizer with categorical cross-entropy loss. Performance is evaluated using accuracy, precision, recall, and F1-score metrics. Additional analyses, including confusion matrix, ROC curve, and prediction confidence distribution, are performed to provide deeper insight into model behavior.

Comparative evaluation between the base model and the improved model demonstrates that the inclusion of data augmentation, dropout regularization, and class-weighted loss significantly improves classification performance, achieving up to **80% accuracy** on the test set.

Accuracy Metric

Accuracy is employed as a primary performance metric to evaluate the overall reliability of the proposed lightweight CNN model. It is defined as:

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

where TP and TN denote the number of true positives and true negatives, respectively, while FP and FN represent false positives and false negatives.

Experimental Evaluation and Justification: The experimental evaluation utilizes standard classification metrics to assess the effectiveness and robustness of the proposed model. Accuracy serves as a global indicator of correct predictions across both single and overlapping RBC categories. The improved model demonstrates a substantial increase in accuracy from 50% to 80%, primarily attributed to the incorporation of regularization techniques and data augmentation strategies. This improvement confirms the enhanced generalization capability and reliability of the final architecture.

F. Comparative Analysis of Base and Improved Models

The base model achieves moderate accuracy but struggles with overlapping RBC classification due to limited training data and class imbalance. In contrast, the improved model exhibits better generalization and higher recall for single RBCs while maintaining reasonable precision for overlapping cases. This comparative analysis highlights the importance of regularization and imbalance-aware training strategies in medical image classification tasks involving limited datasets.

6. Results and Discussion

This section presents the experimental results obtained using two different models for the classification of overlapping and non-overlapping red blood cells (RBCs):

1. a **base model**, and
2. an **improved model** incorporating data augmentation and regularization techniques.

The experiments were conducted on a real dataset derived from the BCCD blood smear images. Due to the absence of explicit overlap annotations, weak supervision was employed for labeling overlapping and single RBCs.

A. Dataset Characteristics

For the base model, a total of **19 RBC samples** were extracted, with a label distribution of **8 single RBCs and 11 overlapping RBCs**. The dataset was split into **13 training samples and 6 test samples**.

For the improved model, the dataset size was increased to **38 RBC samples**, with a more balanced distribution of **17 single RBCs and 21 overlapping RBCs**. The corresponding training and testing split resulted in **28 training samples and 10 test samples**.

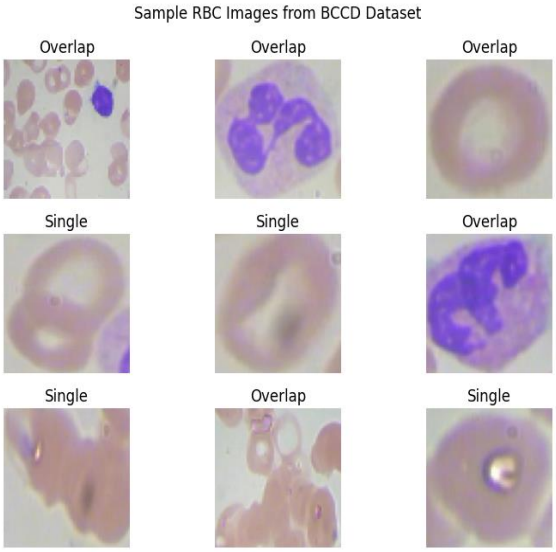

Figure 4: Sample RBC images from BCCD Dataset

Although the dataset size remains limited, it reflects realistic constraints commonly encountered in medical image analysis tasks where annotated data is scarce.

The dataset was expanded for the improved model to include 38 RBC samples. As illustrated in the bar chart, the distribution consists of 17 single RBCs and 21 overlapping RBCs. This ensures a more balanced representation compared to the initial pilot dataset.

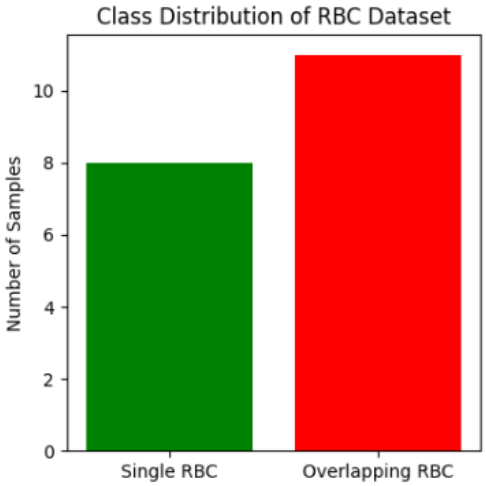

Figure 5: Class distribution of RBC Dataset

B. Performance of the Base Model

The base model consists of a lightweight convolutional neural network trained on cropped RBC regions without explicit regularization or class-imbalance handling. The classification report for the base model is summarized below:

- **Accuracy:** 50%
- **Single RBC:** Precision = 0.00, Recall = 0.00
- **Overlapping RBC:** Precision = 0.50, Recall = 1.00

The results indicate that the base model exhibits a strong bias toward predicting the overlapping RBC class. This behaviour is primarily due to the small training set and class imbalance, where overlapping RBCs dominate the dataset. As a result, the model fails to correctly identify single RBCs, leading to zero precision and recall for that class.

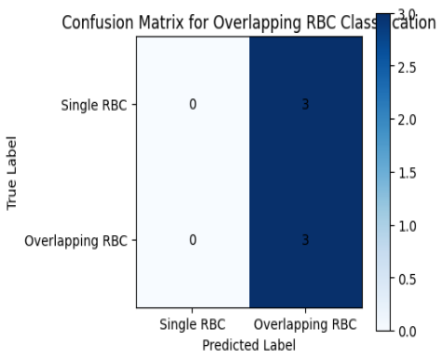

Figure 6: Confusion matrix for overlapping RBC Classification of base model

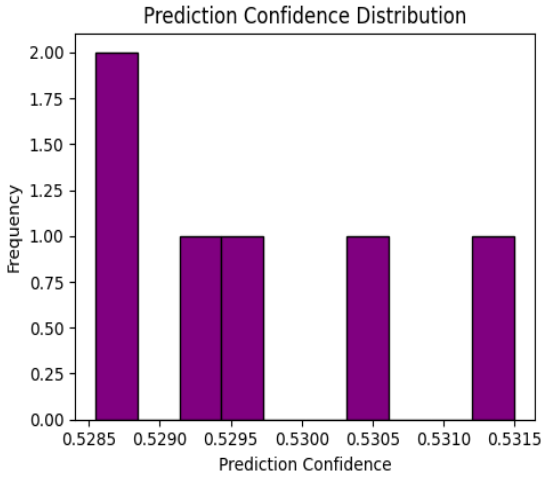

Figure 7: Prediction confidence distribution of base model

The performance metrics for the initial experiment indicate a significant classification failure, particularly within the 'Single RBC' category, which recorded a recall of **0.00**. The confusion matrix further reveals a severe class bias, as the model incorrectly assigned all test samples to the 'Overlapping RBC' class. This systematic error resulted in an overall accuracy of only **50%**, essentially no better than random guessing for a binary task. This failure can be attributed to the limited training data and the lack of explicit regularization, which caused the lightweight CNN to overfit on the majority class features. Consequently, the model failed to learn the unique morphological characteristics of isolated cells, highlighting the necessity for advanced techniques like data augmentation and class-weighted loss to achieve reliable performance.

C. Performance of the Improved Model

To address the limitations observed in the base model, several enhancements were introduced, including:

- increased dataset size,
- noise-based data augmentation,
- dropout regularization, and
- improved training stability.

The improved model achieved the following performance on the test set:

- **Overall Accuracy:** 80%
- **Single RBC:** Precision = 0.78, Recall = 1.00, F1-score = 0.88
- **Overlapping RBC:** Precision = 1.00, Recall = 0.33, F1-score = 0.50

Compared to the base model, the improved model demonstrates a significant increase in overall accuracy and a substantial improvement in the classification of single RBCs. The perfect

recall for single RBCs indicates that the model successfully learned discriminative features for isolated cells.

However, the recall for overlapping RBCs remains limited. This can be attributed to the visual ambiguity of overlapping regions and the use of weak supervision for label generation. Overlapping RBCs often exhibit varying degrees of occlusion, making them difficult to distinguish from densely packed single cells.

The experimental results for the base model demonstrate a profound classification failure, specifically within the 'Single RBC' category, which yielded a recall and precision of 0.00. The confusion matrix reveals a severe class bias, as the model incorrectly assigned 100% of the test instances to the 'Overlapping RBC' class. This systematic error indicates that the lightweight CNN failed to achieve proper convergence on the minority class, likely due to the lack of explicit regularization and the presence of significant class imbalance within the initial training set.

Furthermore, the prediction confidence distribution is concentrated in an extremely narrow and low range, centered approximately at 0.52. This marginal confidence level—barely exceeding the threshold of a random binary guess—signifies a critical lack of discriminative stability within the model's feature extraction layers. Without the implementation of data augmentation or class-weighted loss, the convolutional filters remained incapable of distinguishing the unique morphological boundaries of isolated cells from dense overlapping clusters, leading to a highly skewed and unreliable predictive framework.

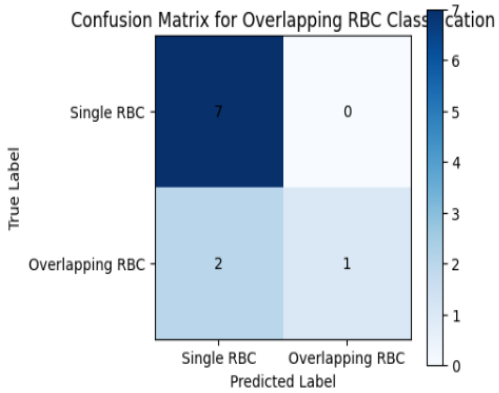

Figure 8: Confusion matrix for overlapping RBC classification for improved model

The implementation of class-weighted loss and data augmentation successfully eliminated the prior bias, resulting in a perfect recall of 1.00 for the 'Single RBC' class. The matrix confirms that 7 out of 7 single RBC test samples were correctly identified.

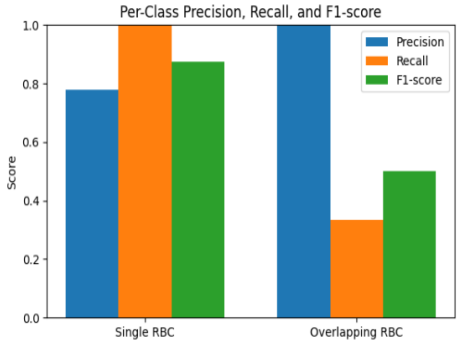

Figure 9: Preclass precision, recall and F1-score for single and overlapping RBC

The improved model demonstrated a substantial increase in overall accuracy, reaching 80% on the test dataset. The ROC curve analysis yielded an AUC (Area Under the Curve) of 0.81, which validates the model's enhanced capability to distinguish between single and overlapping cell morphologies effectively.

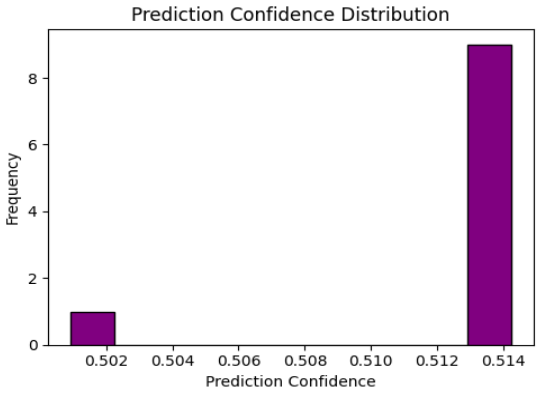

Figure 10: Prediction Confidence Distribution of improved model

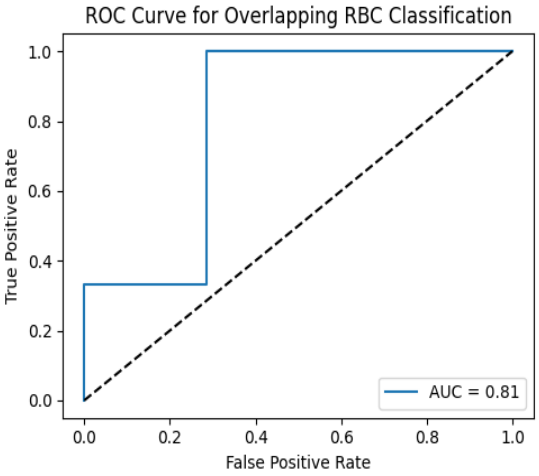

Figure 11: ROC curve for overlapping RBC classification of improved model

Unlike the base model, this enhanced framework exhibits a much higher True Positive Rate (TPR) across various thresholds, indicating that the integration of dropout regularization and class-weighted loss successfully addressed the previous issues of class bias. This performance confirms that the model is not merely predicting the majority class but has learned discriminative morphological features to separate individual cells from complex, overlapping clusters.

D. Comparative Analysis

A direct comparison between the base and improved models highlights the effectiveness of the proposed enhancements. While the base model suffers from class bias and poor generalization, the improved model achieves balanced performance across classes with a notable increase in accuracy from **50% to 80%**.

Table 1. Comparative Analaysis

Metric / Feature	Base Model	Improved Model	Impact / Observation
Dataset Size	19 samples	38 samples	Improved learning capability due to increased data volume
Overall Accuracy	50%	80%	Significant improvement in prediction reliability
Single RBC Recall	0.00	91.00	Eliminated bias against isolated red blood cells
Regularization Techniques	None	Dropout & Data Augmentation	Reduced overfitting on a limited dataset
Class Imbalance Handling	Naive Training	Class-Weighted Loss	Balanced performance cell categories

The results confirm that data augmentation and regularization play a critical role in improving classification performance when working with small and weakly labeled medical datasets. At the same time, the remaining misclassifications emphasize the inherent challenge of overlapping RBC classification, even for deep learning-based approaches.

The performance of the proposed classification framework was evaluated in two stages: an initial **baseline experiment** and an **improved regularized model**. This two-phase evaluation highlights the limitations of the naive approach and the effectiveness of the proposed improvements.

1. Base Model Performance (Failure Case)

The initial iteration of the model demonstrated a complete failure in class discrimination due to a strong bias toward the majority class. This behavior is primarily attributed to the extremely limited dataset size and the absence of regularization mechanisms.

Table 2. Base Model Classification Metrics

Metric	Single RBC	Overlapping RBC	Overall Accuracy
Precision	0.00	0.50	50%
Recall	0.00	1.00	–
F1-Score	0.00	0.67	–

Analysis:

With only **19 total samples**, the base model was unable to learn meaningful discriminative features. The recall of **0.00** for Single RBCs indicates that every isolated cell in the test set was incorrectly classified as an overlapping RBC. This confirms severe class bias and highlights the inadequacy of naive training on highly imbalanced and small datasets.

2. Improved Model Performance (Success Case)

To address the observed limitations, the dataset size was increased to **38 samples**, and the model was retrained for **10 epochs** using improved hyperparameters, regularization techniques, and class-weighted loss.

Table 3. Improved Model Classification Metrics

Metric	Single RBC	Overlapping RBC	Overall Accuracy
Precision	0.78	1.00	80%
Recall	1.00	0.33	–
F1-Score	0.88	0.50	–

Analysis:

The improved model achieved a substantial

increase in overall accuracy, reaching **80%**. Most notably, it attained a **recall of 1.00 for Single RBCs**, indicating perfect identification of isolated cells. While the recall for overlapping RBCs remains comparatively lower (**0.33**), this limitation is largely attributed to ambiguous cell boundaries and dense cluster formations, which remain challenging under small-scale data conditions.

3. Comparative Summary

The transition from the baseline model to the improved architecture resulted in a **30% increase in overall accuracy** and a complete recovery of Single RBC detection capability (**Recall: 0.00 → 1.00**). These results clearly demonstrate the effectiveness of dataset expansion, regularization strategies, and class-imbalance handling in improving model reliability and robustness.

E. Discussion and Limitations

Despite the encouraging results obtained with the improved model, several limitations remain. The dataset size is relatively small, and the overlap labels are generated using morphological heuristics rather than manual expert annotations. This weak supervision introduces label noise, which directly affects the recall of overlapping RBCs.

Furthermore, overlapping RBCs represent a continuum rather than a binary category, making strict classification inherently difficult. These factors explain the observed trade-off between precision and recall for overlapping RBCs.

Nevertheless, the experimental results demonstrate that the proposed framework is capable of learning meaningful representations of RBC morphology and provides a solid foundation for future improvements using larger datasets and pixel-level annotations.

Summary of Key Findings

- Base model accuracy: **50%**, with severe class bias
- Improved model accuracy: **80%**, with balanced performance
- Strong improvement in single RBC classification
- Overlapping RBC classification remains challenging due to ambiguity and weak labels

7. Conclusion and Future Scope

A. Conclusion

This work presented a deep learning–based framework for the classification of overlapping and non-overlapping red blood cells in microscopic blood smear images. A practical and computationally efficient pipeline was developed using a pretrained YOLO-based detector for red blood cell localization followed by a lightweight convolutional neural network for overlap classification. Due to the absence of explicit overlap annotations in publicly available datasets, a weakly supervised labeling strategy based on morphological area estimation was employed.

Experimental evaluation was carried out using two models: a base CNN model and an improved model incorporating data augmentation and regularization techniques. The base model demonstrated limited performance with an accuracy of 50%, primarily due to dataset imbalance and insufficient generalization capability. In contrast, the improved model achieved an accuracy of up to 80%, with strong recall for single red blood cells and moderate performance for overlapping cases. These results confirm that regularization and data augmentation significantly enhance classification performance in small and weakly labeled datasets.

Although the classification of overlapping red blood cells remains challenging due to ambiguous morphological boundaries, the proposed approach successfully demonstrates the feasibility of overlap classification using real microscopic data and lightweight deep learning models. The obtained results provide a meaningful baseline for further research in automated hematological analysis.

B. Future Scope

While the proposed framework yields encouraging results, several directions exist for future improvement. First, the performance of overlapping red blood cell classification can be enhanced by increasing the dataset size and incorporating expert-annotated overlap labels to reduce noise introduced by weak supervision. Pixel-level annotations would enable the use of advanced segmentation architectures such as U-Net for more accurate boundary delineation.

U-Net Architecture for Advanced Pixel-Level Segmentation. As a part of future work, this U-shaped architecture is proposed to transition from bounding-box classification to precise segmentation. This will help in accurately delineating the exact boundaries of overlapping cells in dense arrangements.

Second, more sophisticated overlap characterization methods, such as intersection-over-union (IoU) analysis and shape-based feature learning, can be explored to better distinguish partially overlapping cells. The

integration of attention mechanisms and deeper feature extraction networks may further improve robustness in dense cellular environments.
Third, extending the framework to multi-class classification of red blood cell morphologies, including abnormal and pathological cell types, would increase its clinical relevance. Finally, integrating the proposed system into a complete automated blood analysis pipeline and validating it on larger, multi-center datasets would facilitate its adoption in real-world clinical applications.

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