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RESEARCH ARTICLE

Deciphering Blood Cells - Method for Blood Cell Analysis using Microscopic Images

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Abstract

Blood cell analysis of RBC and WBC from smear images is crucial for identifying disorders caused by abnormal blood cells, such as leukemia, anemia, frequent infections, and other diseases, including cancer. Pathologists find it challenging to distinguish between abnormal and normal cells in smear images. Manually counting each element is tedious and prone to human errors. Image processing techniques are used to enhance the smear image quality before segmentation. Identifying and segmenting smear images remains a challenge for pathologists. In this research, we propose an approach for identifying and segmenting smear images, followed by classifying normal and abnormal cells. This work implements Mask CNN for segmentation and two different algorithms: EfficientNet-B3 and DenseNet-121 for classification. The Mask CNN model's results for identification and segmentation are evaluated using mAP and IOU. For RBC, the best mAP value is 0.52 and IOU is 0.45. For WBC, the best mAP value is 0.56 and IOU is 0.52. Classification results outperform previous studies, with experiments on six different classes based on RBC and WBC cell normality and abnormality. The average overall accuracy for EfficientNet-B3 is 95% and DenseNet-121 is 97%, respectively.

Keywords: RBC (Red Blood Cells), WBC (White Blood Cells), Mean Average Precision (mAP), Intersection of Union, Classification, Segmentation, AUC.

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1. Introduction

Various laboratory examinations are conducted by pathologists on blood to know about morphological properties of blood which is termed blood cell analysis. Inside the bone marrow the blood cells are developed. Bone marrow is characterized as soft and spongy in centre of bones [1]- [3]. Around 95% of the body's blood cells is produced from the bone marrow. The total blood volume of an average adult is about 6 L. Leukocytes and thrombocytes only comprise about 1% of the blood volume whereas plasma being the largest portion of the blood and is about 55%. Blood is a specialized fluid that circulates in an extracellular fluid called plasma. Plasma typically consists of about 92% of water, 7% protein and 1% is a combination of other solutions. The three main types of blood cells are erythrocytes (RBCs), leukocytes (WBCs) and thrombocytes. According to the study of university of Rochester medical centre, for a normal human being the count range of each blood cell is as follows: WBC count scales from 3,300 to 8,700 and RBC count

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scales from 3.93 to 5.69 million cells per cubic millimeter (million/mm^3). Normal range of blood count for males above the age of 15 is about 13.0–17.0 g/dL and for females it is 11.5–15.5 g/dL. The cell shape of RBCs is bi-concave disc shape, in terms of diameter its size varies from 6–8 μm, about 120 days is its approximate life span. Unlike WBCs, due to the presence of hemoglobin on maturity, red blood cells do not have a nucleus, these cells appear red in colour. WBCs size varies from 12–17 μm in diameter and they are irregular in shape. The existence of WBC is around 12–20 days. Due to the absence of any pigment the WBCs cells are colourless. A Single type of RBC is present. whereas WBC has several types of mainly T lymphocytes, B lymphocytes, neutrophils, monocytes, basophils and eosinophils [4]- [8].

To determine the number of leukocytes and erythrocytes in blood there are numerous tests, along with hemoglobin's concentration, volume and sedimentation rate. In addition, there are various test that are used to classify to which blood group the cells belong to and other test focus on the morphological details of the cells. To detect various diseases and to get overall information about a person's health, a complete blood test is done. During the test, the RBC analysis is performed. The RBC's and WBCs cell analysis plays an essential role in detecting the diseases and disorders that are caused. Shape, colour, inclusions and arrangements are considered during the analysis of blood. Usually hematologists analyse the blood smear images manually through microscopic views. Manual process of visualising the blood smear slides takes quite a long time and requires domain expertise for accurate prediction. In recent years the technology improvement in the domain of artificial intelligence and medical imaging has come up with new tools and technology to help the hematologists to make their tedious process easier. To detect and analyse the blood cell images, the most recent technology uses deep learning approach which does not fail to give accurate results on classification tasks.

The work of [7] proposes a pipeline to detect different kinds of diseases. Helping the hematologists in counting the blood cells, the author of this journal [2] comes up with an approach to identify shape of the RBCs image has abnormal and normal by using Form Factor and uses feature descriptors as perimeter and Area. The paper focuses on reducing the intensity of human power and his efforts involved in non-automatic classification of RBCs. Method to segment and classify RBCs from blood smear images and focuses on resolving the problem of cell overlapping and data imbalance problems that usually occur while dealing with multiple classes in deep learning [12]. By using smear images, a fully automated model is developed for identification and classification of WBC cells [8]. Few other methods and proposals have evolved over time that try to classify, detect, count, segment, and morphological characters of the blood cells.

This work deals with various preprocessing steps used mainly Conversion of RGB image to Binary Image, CLAHE (Contrast Limited Adaptive Histogram Equalization) technique on Binary Image, Gaussian Blur and Erosion-Dilation Technique, RBC Edge Detection Methods, Contour extraction on the image, Drawing Bounding Boxes and Masking technique for Segmentation is used. Masking technique is an image technique in which we try to modify a large image by defining a small piece of it.

2. Related work

Over decades there has been continuous research and extensive work done on analysis of blood cells in various domains like computer vision, image processing and artificial intelligence. Study on blood cell count and its characteristics as shape, colour, size etc. help in better detection and evaluation of the disease that a person is suffering from. To recognize various kind of diseases, Helping the hematologists in counting the blood cells, the authors of [7] proposes a pipeline that follows two steps: Initial step is to detect different kind of nucleus of WBC followed with the next step where the number of WBC and the abnormal WBC nucleus is counted. In order to identify different type of nucleus, the colour image is converted to grey scale followed by thresholding segmentation, image optimization and noise removal technique. After applying the previously mentioned techniques morphological operation is applied to smoothen out the nucleus border which determines the segmentation and contour of the nucleus. To achieve the lateral phase of the proposed methodology. In order to remove noise median filtering is used, optimized image is obtained on using bitwise OR operation. During the segmentation process, masking technique is used to get the masked image, with a structuring element, on the image matrix the morphological opening is performed, the connected area is labelled, combined number of nucleus is obtained and size of each Nucleus is stored. The proposed framework in this paper has achieved 85% of accuracy with usage of segmentation and edge detection method in identifying different type of WBC. Unlike the usage of conventional methods, The paper talks about implementation of a new method to detect the edges of nucleus and also provides an operation to identify abnormal WBC nucleus, Along with the size of WBC nucleus and counting of total number of WBC.

When it comes to blood cells analysis and classification, time is the major constraint. One such paper that focus on time constraint in classification of blood cells is normal and abnormal red blood cell recognition using image processing [2]. It proposes a pipeline method for detection of the blood cells, original oriented image samples of normal and pathological

(abnormal) cells on smear images are automatically generated. The image further under-goes pre-processing steps where the grey scale image is further processed has RGB image, on which thresholding method like Otsu is applied then followed by image complementing method, image cropping, hole filling, and feature extraction using form factor. All together SVM model was introduced to classify normal and abnormal blood cells and achieved the results based on the matrices like sensitivity, specificity and accuracy to its respective values: 100%, 89.47% and 94.12%.

On classifying blood cells usually, there occurs a problem of cells overlapping, the paper [12] comes up with a methodology where it initially follows the method RBC colour normalization which is very prominent while training a model as the colour feature of the cells plays a greater role. Followed to RBC colour normalization, RBC contour extraction is applied and CLAHE is applied to enhance the contrast and make the foreground distinguishable from background. Finally, to extract all the regions Otsu thresholding and contour findings are used. The third step in the pipeline is overlapping cell separation, the most reliable methods for cell separation are based on distance transforming and ellipse fitting. This method finds concave points and use direct ellipse fitting on the set of detected convex curves to estimate the shape of a single RBC. The paper uses Efficient Net-B1 model.

Jambhekar, N.D.: Red blood cells classification using image processing. Science Research Reporter [6] emphasis on time-consumption, wrong interpretation, human error and proposes a pipeline where the input images are taken and are pre-processed. The sub images are obtained, and the features are extracted for the same. The methodology that this paper [6] tries to produce a model that can identify the normal and abnormal cells of RBC using image processing and CNN techniques which results with an accuracy of 98% it is able to categorize different categories of RBC's abnormal cells into 9 classes. There are many ways to differentiate and classify RBC's and one such paper Deep learning-based phenotyping of breast cancer cells using lens-free digital in-line holography. In [19] talks about classifying through the shape of RBC's which basically involves edge detection and segmentation, The authors of [13] proposes a WBC segmentation pipeline on coloured images, with a techniques such as scale-space filtering for nucleus extraction and for cytoplasm extraction it uses watershed clustering techniques. Approaches in this paper uses two edge detection methods: Gradient and Laplacian.

After extracting features, identification and classification of cells, then updating the count. All these steps are involved in the traditional way of image binarization. Methods as discussed in [13], [14], [10] like "watershed", "Gabor filter", "Active contour" and "Gradient vector flow" are used to count the blood cells too [4]- [6], [17], [18], [23], [24]. Among various research's, the [16] comes up with a method for equality analysis of covariance matrix in Gobar-filtered holographic images. This paper aims to provide an approach for red blood cells to automatically select a linear or nonlinear classifier. This journal produces an Off-axis digital holographic and 3D morphology statistical methods to classify the RBC's. The RBC images are reconstructed numerically while watershed marker-controlled algorithm is used for segmenting the inner part of the cell. Followed by feature extraction method, from Gabor-wavelet-filtered RBC 14 features are determined. Followed by a non-linear or a linear classification method that is evaluated based on equality of covariance matrices on multivariate test. Results says that it has zero miss classification rate. To examine RBC-related diseases and to test the drugs that are related to screen compounds of RBC's, to classify these the mentioned algorithm for classification can be used. The work in ref [3] uses ALL-IDB image dataset to develop an approach where it remotes the entire cell and then divides the cytoplasm and nucleus. Nucleus selection makes use of the observation of the paper [11] and for thresholding operation, Otsu is followed whose inference is taken out from the journal [8]. This approach is essential to analyze every cell unit in detail. Shape, colour, texture and different features, these are extracted from each cell component using background pixel removal approach. For detection of leukemia, different classification models are trained on these feature set to determine which one is most suitable. Outcomes justify automatic identification and classification of leucoplasts is efficiently identifying WBCs present in an image. The feature extraction method that is proposed in the paper is extracting features from cropped images in both chromatic and texture features.

3. Data acquisition

The dataset is collected from various sources i.e., Mendeley image dataset, Cancer image archive and from different open-sources. Open-source sites include smear cell (RBC) dataset, smear cell (WBC) dataset. The RBC dataset contains 738 smear images, which also have annotations of the cells as normal or abnormal (Macrocyte, Microcyte, Spherocyte, Target cell, Somatotype, Ovalocyte, Teardrop, Burr cell, Schistocyte, Hypochromia, Elliptocyte) cells of smear images. The different types of abnormal cells also help to identify the distinctive types of diseases that occurs from the blood.

Mendeley image dataset contains the single Normal WBC cell in each smear image. These images are obtained using the analyzer Cella-Vision DM96 in the Core Laboratory at the Hospital Clinic of Barcelona. The dataset consist of various types of cells such as basophils, neutrophils, lymphocytes, eosinophils, monocytes immature granulocytes

(metamyelocytes, promyelocytes and myelocytes), erythroblasts and platelets or thrombocytes. The default image sizes are 360 × 363. Cancer image archive dataset contains the single abnormal WBC cell in each smear image. The dataset contains of total 18,365 smear images, which are further classified into 10 different classes of cells. These images are in the image size of 400 × 400. There are 14,712 WBC cells and rest belongs to other classes. In-house dataset is prepared by combining the overall WBC cells into 3:2 ratio. The dataset is split into 4 classes as WBC Normal, WBC Abnormal, RBC Normal and RBC Abnormal.

4. Data Preprocessing

Once the data is acquired, the smear images are preprocessed into a grayscale image and further transferred into other preprocessing steps [9]. Once all the images are similar to each other with respect to the color intensity and ratio, then only further processing steps on the image are applied [7]. In this approach various different conversion methodologies have been implemented such as BGR to GRAY, BGR to LUV, BGR to LAB [1]- [5]. The method which produced better results as compared to other methods was BGR to LAB. The intensity of the cell in the smear image has substantial difference.

The CLAHE technique improves the local contrast by enhancing the definitions of edges in each cell of a smear image, Since the focus is on the edges of the cells to easily distinguish between background and cells in the image [9]. Combining the CLAHE technique with L channel (Lightness) since it have more contrast than other channels of binary image. Further the image is normalised, where the edges of the each cell pleasingly contrasted and differentiated from its background [16].

A contour technique is processed on edge detected image to find the boundary values of each individual cells [12]. The contour extraction methodology works with the image and process it with contour retrieval and contour approximation mode method which helps in joining all the continuous points of cells edges having same color intensity and ratio. Once the contour values are extracted, those values are further drawn on the original image. To identify each cell individually, drawing bounding boxes around each cell is essential. This can be done with the values of the of edges, contours, thresholds which have been acquired from previous image processing techniques. To draw the bounding box around each cell minimum area rectangle method is used [2].

Once the classification of the dataset have been done, the focus is on segmentation of the images. For WBC cells, each cell is cropped according to there bounding boxes and mapped as a separate smear image. For the segmentation, masking technique is used to get the area of interest in an image, i.e. WBC cell. Further creating a mask on the image and then drawing ellipse on the masked image. The ellipse is the WBC cell whereas the background is masked [12]. For RBC cells smear images annotation is performed for the segmenting the cell as normal and abnormal. A CVAT tool is used for the annotating the smear images of RBC dataset and annotations are saved in COCO format. Further drawing ellipse on the masked image. The ellipse is RBC cell whereas the background is masked. For better understanding refer the Figure 1.

Table 1: Open-source RBC in-house dataset collected.

RBC Cell Type	**Smear Image Type**	**RBC cells count**
Normal	142	6330
Macrocyte	67	689
Microcyte	11	461
Spherocyte	63	3449
Target Cell	117	2753
Stomatocyte	42	1996
Ovalocyte	33	2140
Teardrop	32	307
Burr Cell	26	785
Schistocyte	35	897
Hypochromia	32	1063
Elliptocyte	32	1053
Uncategorized	3	184

Table 2: Open-Source WBC in-house dataset collected.

WBC Cell Type	WBC Normal Cell Count	WBC Abnormal Cell Count
Basophil	1218	78
Eosinophil	3117	422
Monocyte	1420	1750
Lymphocyte	1214	3924
Neutrophil	3329	8538

To eliminate the unwanted noise in the image, the erosion-dilation and Gaussian blur techniques [21] were significantly useful and effective. Erosion technique erodes away the boundaries of the cells and removes small-scale details such as unwanted edges. After erosion, dilation is used to dilate the eroded region [2]. Once the process is done, threshold values are calculated for the further process. There are various edge detection techniques such as Laplacian edge detection, Canny edge detection [12] and Sobel edge detection technique. Since our focus is on RBC and WBC images where the detailing of each cell edge must be captured. The Canny edge detection [19] technique performed well with this type of image dataset and accepts binary images as input, and it is also a multistage algorithm due to which the results are much better than Laplacian and Sobel edge detection techniques. In this technique, the upper and lower limits of the threshold values automatically proceeds and detect all the cell edges with satisfactory accuracy.

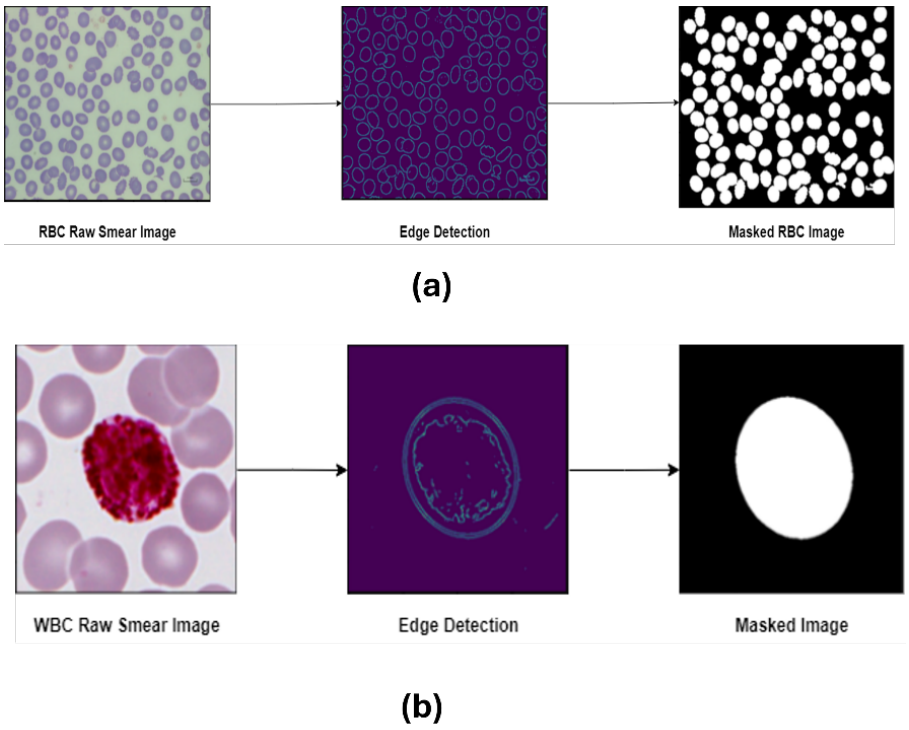

Figure 1: Masking on smear images (a) RBC (b) WBC.

5. Methodology and experiment

There are several approaches and experiments that have been performed to classify and segment the blood cells (RBC's and WBC's) from smear images. For segmentation of blood cells, the datasets are prepared in specific format. Whereas it contains the original image and masked image with a specific image id. This dataset is further passed through a specific model for identification and segmentation of blood cells. The ref of [5] model is trained for segmentation and identification of blood cells. The Resnet50-v1 model is trained on coco dataset, which has been used as pre-trained model for Maskrcnn with image-net as source. This Resnet50-v1 uses the VOOC format for bounding box and image is normalized to 512×512 for training [22]. As a pre-processing, the images are normalised and resized into 256×256 and batched. The dataset is split into 9:1 ratio for train and validation purpose. As shown in Figure 2, the original image

and masked image of smear image is passed through models and this models first identify the bounding box around the mask images, creates the bounding boxes in the pascal format as $(x, y, x_{\max}, y_{\max}, x_{\min}, y_{\min})$ and class names are been assigned to unique class id's and labels of smear image is stored. Once after the training the models are been evaluated based on the loss functions and Mean Average Precision (mAP) scores with threshold as 0.5. The loss functions of the of model is defined as overall loss (L), object loss (L_{Obj}), mask loss (L_{mask}), bounding box loss (L_{BB}). Which is defined as $L = L_{\text{Obj}} + L_{\text{mask}} + L_{\text{BB}}$.

$$l_{\text{box}} = \lambda_{\text{coord}} \sum_{i=0}^{S^2} \sum_{j=0}^{B} I_{i,j}^{\text{obj}} (2 - w_i h_i) \times \left[(x_i - x_i^j)^2 + (y_i - y_i^j)^2 + (w_i - w_i^j)^2 + (h_i - h_i^j)^2 \right]. \tag{1}$$

$$l_{\text{cls}} = \lambda_{\text{class}} \sum_{i=0}^{S^2} \sum_{j=0}^{B} I_{i,j}^{\text{obj}} \sum_{c \in \text{classes}} \left[p_i(c) \log\left(p_i(c)\right) \right]. \tag{2}$$

$$l_{\text{obj}} = \lambda_{\text{noobj}} \sum_{i=0}^{S^2} \sum_{j=0}^{B} I_{i,j}^{\text{noobj}} (c_i - \widehat{c}_i)^2 + \lambda_{\text{obj}} \sum_{i=0}^{S^2} \sum_{j=0}^{B} I_{i,j}^{\text{obj}} (c_i - \widehat{c}_i)^2. \tag{3}$$

In (1)–(3), the values are as follows lambda is the loss coefficient of $\widehat{x}$, $\widehat{y}$ which are also the grid coordinates and h, $\widehat{w}$ are the representing the height and width of the grid. If the grid at (i, j) covers the targets, then the value of $I_{i,j}^{\text{obj}}$ is equals to 1; otherwise, the value remains 0. $p_i(c)$ represents the target probability category, and $p_i(c)$ represents true values of the category. The total number of categories is equal to the length of both the categories [1], [15]. Classification results on blood cells are shown in Table 3, whereas Efficient Net-B3 is given in Table 4.

$$mAP = \frac{1}{N} \sum_{i=1}^{N} AP_i. \tag{4}$$

mAP = mean Average Precision

$$IoU = \frac{TP}{TP + FP + FN}. \tag{5}$$

IoU – Intersection of Union
TP = True Positive
FP – False Positive
FN – False Negative

6. Model evaluation and result

An inhouse-house dataset is prepared by combining the RBC's and WBC's datasets into 3:2 ratio. The experiments have been carried out on two different models which are DenseNet-121 and Efficient Net-B3 [20] for classification of cells into Normal and Abnormal types of RBC and WBC cells. All the experiments are conducted on three different datasets. Whereas models are trained on RBC and WBC datasets separately to identify only normal and abnormal cells. The experiment is carried out on the 2 models for four and six different classes (RBC, WBC, WBC Normal, WBC Abnormal, RBC-Normal, RBC Abnormal).

The RBC smear images were annotated with the help of CVAT tool and were further divided into 8:1:1 for training, testing and validation in the form of COCO dataset. The smear images are passed through Mask RCNN model on imagine dataset with COCO data format and post-processed the masked json file into changing its configuration and retained on it. The results of the trained models with confusion matrix and AUC are shown in Tables 5 and 6.

Table 3: Classification results on blood cells.

EfficientNet-B3					
Tag	**Accuracy**	**Sensitivity**	**Precision**	**F1-Score**	**Specificity**
RBC-Normal	0.9575	0.93452381	0.73364486	0.82199	0.960195531
RBC-Abnormal	0.96063	0.872685185	0.979220779	0.92289	0.993150685
WBC-Normal	0.99938	0.996539792	0.989028820	0.99827	0.99236
WBC-Abormal	0.97032	0.99230	0.96382	0.98437	0.97323
DenseNet-121					
Tag	**Accuracy**	**Sensitivity**	**Precision**	**F1-Score**	**Specificity**
RBC-Normal	0.96125	0.911765	0.712644	0.80000	0.965847
RBC-Abnormal	0.97000	0.930233	0.956938	0.943396	0.984615
WBC-Normal	0.97323	0.98323	0.99034	0.97662	0.98112
WBC-Abormal	0.96998	0.98323	0.99245	0.98789	0.97213

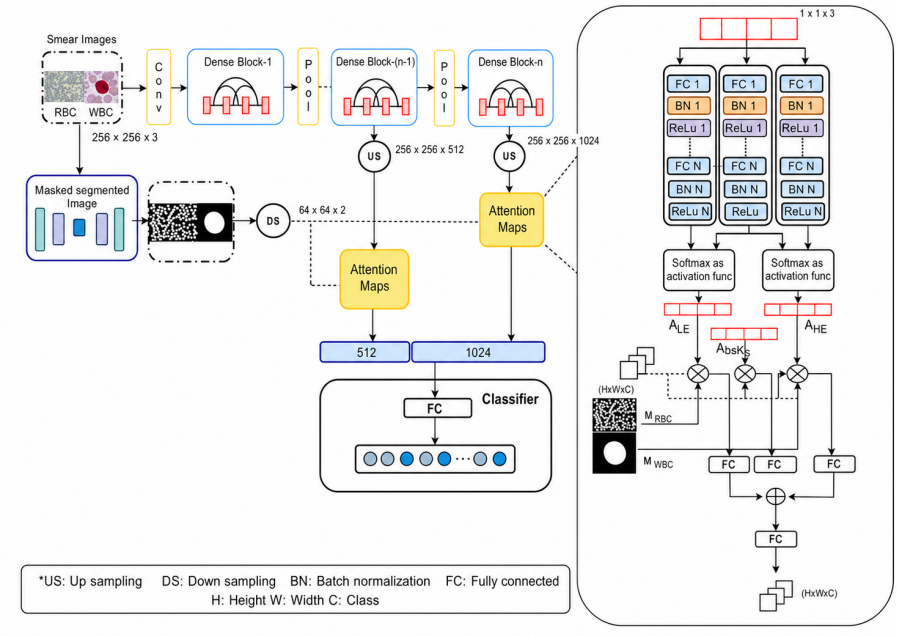

Figure 2: The model architecture includes identification and classification algorithms. DenseNet-121 and EfficientNet-B3 classify WBC and RBC smear images as Normal or Abnormal.

Table 4: EfficientNet-B3.

Tag	**EfficientNet-B3**				
	Accuracy	**Sensitivity**	**Precision**	**F1-Score**	**Specificity**
RBC-Normal	0.94125	0.988095238	0.643410853	0.77934	0.93575419
RBC-Abnormal	0.7675	0.143518519	0.96875	0.25	0.998287671
WBC-Normal	0.99875	0.993079585	0.983467	0.99653	0.983254
WBC-Abormal	0.993435	0.984353	0.994353	0.9911771	0.9707980
RBC	0.98375	0.956666667	0.995838	0.97785	0.9923727
WBC	0.99188	0.9902343	0.987166831	0.99354	0.978333333

Table 5: Classification result on metric AUC.

Model	**Data**	**Normal**	**Abnormal**
EfficientNet-B3	Test-400	0.98057309	0.97826615
	Val-200	0.95880977	0.95465177
DenseNet-121	Test-400	0.97222559	0.97334871
	Val-200	0.96452703	0.96049896

Table 6: Blood cells segmentation results.

Blood Cell	**No. of Experiment**	**Training mAP**	**mAP**	**IOU**	**Image Size**
RBC	Best-1	0.5195	0.5206	0.4166	256×256
	Best-2	0.4939	0.4939	0.3649	256×256
WBC	Best-1	0.6922	0.5650	0.5289	896×896
	Best-2	0.6305	0.5450	0.4780	256×256

7. Conclusion

In recent years, the healthcare domain has evolved and requires technical improvements in terms of automation for identifying and classifying the blood cells (RBC, WBC) through a smear image. Pathologists face issues while identifying and classifying blood cells into normal or abnormal blood cells. The proposed approach uses the in-house dataset on which a modern architecture is implemented for identification and segmentation using Mask RCNN and classification is implemented using EfficientNet-B3 and DenseNet-121 which outperforms the previous work in terms of time efficiency and accuracy. Finally, the proposed work helps the pathologist to solve these issues in the blood cell analysis and identify diseases through it is useful to minimize the human errors.

Declarations and ethical statements

Conflict of interest: The authors declare that there is no conflict of interest.
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Artificial Intelligence usage statement: During the preparation of this manuscript, the authors utilized ChatGPT solely for language refinement and grammatical corrections. The authors carefully reviewed and revised the generated content and take full responsibility for the accuracy, integrity, and originality of the final manuscript.
Availability of data and materials: The data and/or materials that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Sandeep Kumar Aazad: Conceptualization, Implementation, Methodology, Writing–Original draft, Review & Editing. **Taniya Saini**: Implementation, Methodology, Writing–Review & Editing. **Ashok Ajad**: Implementation, Methodology, Visualization, Writing–Review & Editing. **Kritika Chaudhary**: Implementation, Visualization, Writing–Review & Editing. **Ebrahim E. Elsayed** : Formal analysis & Validation.

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