

A SURVEY ON VARIOUS APPROACHES FOR SEGMENTATION AND CLASSIFICATION OF WHITE BLOOD CELLS

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Abstract

Traditionally, White Blood Cell identification is performed by experienced pathologists manually. Peripheral blood smear analysis is a laboratory procedure that helps in diagnosis of various pathological disorders such as malaria, anemia, leukemia, etc. This process is performed at a microscopic level and therefore is required to be precise. Manually performing microscopic evaluation is a challenging task. This task can be automated with the help of computer-aided systems. This work discusses various methods being used in a variety of cellular image segmentation and classification tasks with the field of the survey being concentrated on leukocyte segmentation and classification.

Keywords: Segmentation, Classification, Leukocytes, White Blood Cells, Deep Learning

Introduction

The human body is as unique and complex as it can get. Being a highly organized structure that is made up of cells that constitutes tissues to the largest organs in the body, the anatomy is difficult and yet very beautiful at the same time. One such important part of the human body is blood or medically termed as peripheral blood fluids. It is responsible for carrying and supplying oxygen to the whole human body. It is made up of Red Blood Cells viz RBCs (also termed as Erythrocytes), White Blood Cells viz WBCs (also termed as Leukocytes), plasma (Sapna and Renuka, 2017) and Platelets

(also termed as Thrombocytes). This study is concentrated on Leukocytes.

WBCs protect our body against both infectious diseases and foreign invaders. They are the ones responsible for boosting immunity in humans. There are two main categories of WBCs: Granulocytes and Agranulocytes. Granulocytes are further categorized into Basophils, Neutrophils and Eosinophils. Agranulocytes are further categorized as Lymphocytes and Monocytes. Figure 1 shows different categories of WBCs. An individual's health condition is monitored based on the count of

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each category of WBC. Pathologists and lab technicians perform manual identification, counting and grouping of the leukocytes, which is a tedious task and is prone to errors. Computer-aided systems come to the rescue in automating this manual task. Leukocyte Segmentation plays a critical part in smear image analysis. The segmentation results obtained can help in classifying the images as it is dependent on the features extracted from the segmented regions of interest.

For diagnostic purposes, various research groups have continued to work on different aspects of automation of peripheral blood image sample analysis. The basic procedure followed for identification and analysis of WBCs in peripheral blood smear samples is listed below:

- a. Staining the blood samples
- b. Preparing the Dataset
- c. Applying the Pre-processing Techniques
- d. Segmentation of WBCs
- e. Classification of the WBCs
- f. Counting the number of WBCs in a sample

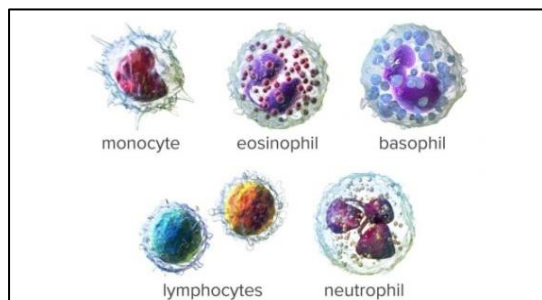


Figure 1. Types of White Blood Cells

The methodology followed by traditional computer-aided devices to perform peripheral blood smear analysis includes the steps such as Image Acquisition, Pre-processing, Segmentation, Feature Extraction and Selection, and finally Classification. Convolutional Neural Networks (CNN) architecture, a deep learning methodology can extract the essential features from certain parts of an object and can perform object recognition (Zeng *et al.*, 2019). Based on the available works of many researchers, a survey has been carried out on many different techniques that are in use, specific to the biomedical domain. In this paper, we highlight the different techniques that can be opted to perform cell segmentation, classification and counting.

Literature Review

This section provides a detailed review on the different types of staining techniques used for generating the dataset, description of the publicly available datasets for WBC segmentation and classification and the techniques which have been proposed in recent times for segmentation, classification and counting of WBCs.

Staining Techniques

Preparation of peripheral blood film slide is a very crucial part of obtaining the leukocytes samples. Staining is the technique that is employed to ensure accurate and reliable samples. Slide preparation is done by trained laboratory personnel, who can ensure quality slides for microscopy. The thickness of the smear also plays an important role. After the slides are smeared, they are air-dried before dipping them in the stain solutions. The dried stain is dipped in absolute methanol or ethanol and then stained with the Rowmanosky stain. This is a mixture made from acidic and basic dyes that provide different stains for different cellular components (Adewoyin, 2014). It provides a fine distinction between the components involved. The different components of this stain are - Azure B, Eosin Y, Azure A, Azure C and Methyl violet. The neutrophil granules are coloured by Azure complexes, eosinophil by the acid particles of the solution and basophils by the basic dye particles, as they are acidic in nature (Peripheral Smear Staining). There are six types of Rowmanosky stain solutions.

Leishman's stain

It is a commonly used staining technique where Polychrome methylene blue acts as a base and eosin as acid (Adewoyin, 2014). The slides are kept in the stain solution for two minutes. After staining the slide, it is diluted with distilled water and then kept drying for about eight minutes. It is then washed with distilled water and air-dried for microscopy (Peripheral Smear Staining). This technique provides better visualization for WBC. Figure 2 represents the Leishman's stained sample.

Wright's Stain

Wright's stain is made up of a mixture of eosin (acts as an acid) and methylene blue dyes (acts as a base) (Adewoyin, 2014). It is normally used for staining different types of blood cells. Bone

marrow, urine and blood smear samples are stained using this technique. Figure 3 represents Wright's stain sample. The cytoplasm being basic is stained orange pink and other cellular components have stained a variation of blues.

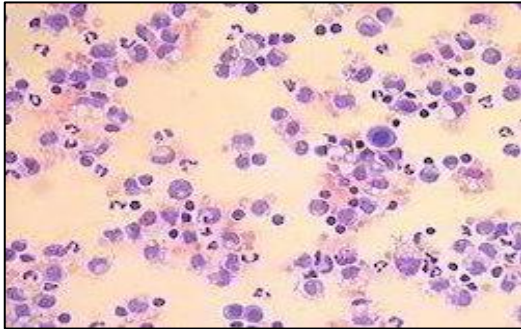


Figure 2. Leishman's Stain Sample

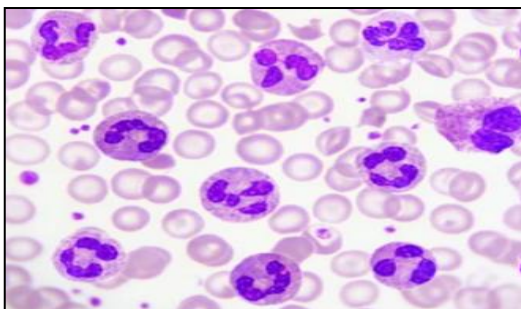


Figure 3. Wright's stain sample

Giemsa's stain

Giemsa's stain is a nucleic acid stain named after the German bacteriologist Gustav Giemsa. It is used in the diagnosis of malaria and intracellular parasites. This stain is generally used at 1:10 dilution. Figure 4 shows the slide microscopic view for Giemsa's stain. Giemsa's stain requires more time to be prepared than Leishman's stain.

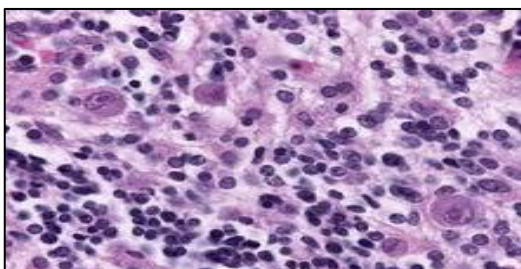


Figure 4. Giemsa's stain sample

Field's stain

This stain named after physician John William Field has two parts - stain A containing methylene blue and Azure A and stain B containing eosin Y. Figure 5 has a sample image taken of the slide prepared with this technique. Normally used when films are smeared thickly to easily discover malarial intruders. This stain provides rapid specimen processing.

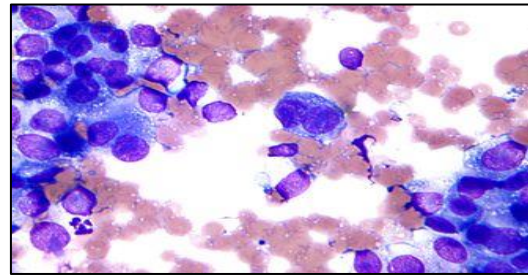


Figure 5. Field's stain sample

Jenner's stain

Jenner's stain is also known as methylene blue eosinate and it is used to stain the nuclei in blood smears with a dark blue shade. It is recommended to prepare a new Jenner solution after each use. It is also used for bone marrow sample staining. It produces results that are similar to Giemsa's stain and can be used to separate bone marrow cells. Figure 6 represents Jenner's stain sample.

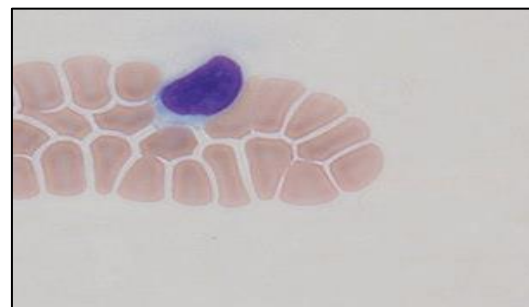


Figure 6. Jenner's stain sample

May Grunwald Giemsa (MGG) stain

MGG stain is used for staining blood, bone marrow and cytological samples. It has applications like morphological inspection and counting the blood cells. It is a mixture of methylene blue, azures, and eosin. When used with Giemsa, the azure components stand out more as represented in Figure 7. The solution is dark violet in colour.

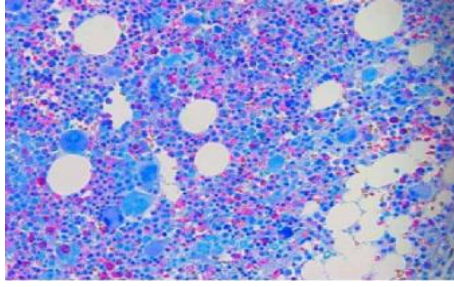


Figure 7. MGG stain sample

Dataset

For designing a robust computer-aided system a diverse image dataset is required. To test the accuracy of the segmentation and classification model there are few publicly available datasets. Leukocyte Image Segmentation and Classification (LISC) Database (Rezatofghi and Soltanian-Zadeh, 2011) consists of 376 blood smear image samples. Ground truth segmented image is available only for 250 images. These image samples were collected from the healthy normal individuals. Gismo-Right staining Technique was performed on the blood smear. These WBCs were manually classified by considering different factors into account such as size, textures etc accordingly into 5 different categories by haematological experts. A sample blood smear image along with the ground-truth has been shown in Figure 8.

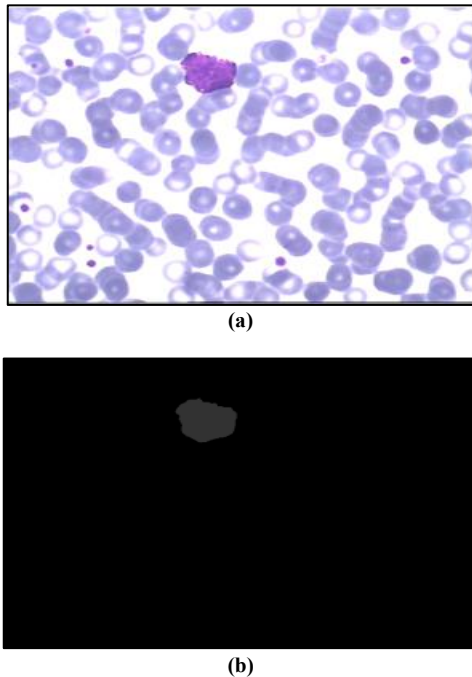


Figure 8. (a) Sample blood smear image from LISC Database. (b) Ground Truth Image

ALL_IDB Dataset (Labati *et al.*, 2011) is made up Acute Lymphoblastic Leukemia (ALL) images and is subcategorized into ALL_IDB1 and ALL_IDB2 Dataset. The dataset also differentiates the blood image samples into normal healthy cells and Acute Lymphoblastic Leukemia cells. Figure 9 shows the ALL_IDB1 dataset Images. ALL_IDB1 Dataset contains peripheral blood smear image samples with multiple white blood cells. The ALL_IDB2 Dataset consists mainly of single WBC cell image samples. Figure 10 displays the ALL_IDB2 Dataset images. In total, there are 108 Images and 260 Images in ALL_IDB1 and ALL_IDB2 dataset respectively.

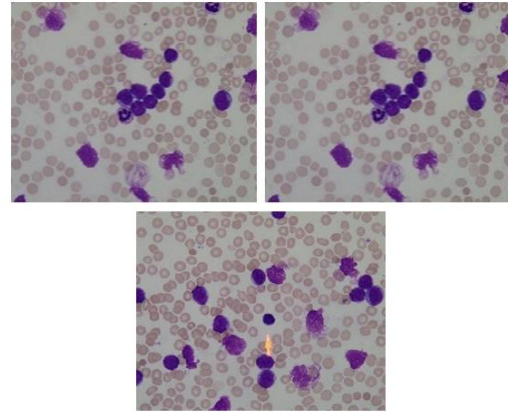


Figure 9. Sample images from ALL_IDB1 Dataset

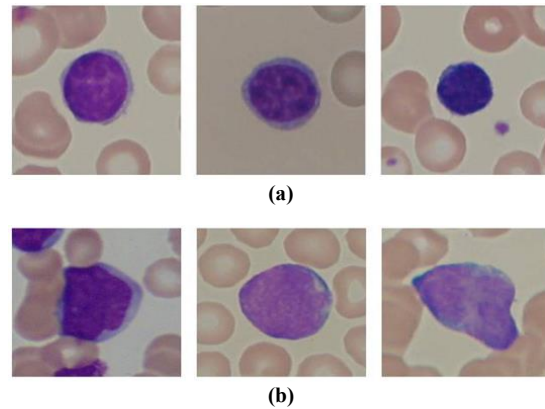


Figure 10. ALL_IDB2 DATASET Images (a) Represents blast cells from ALL patients. (b) Healthy cells from Non ALL patients

Dataset 1 (Zheng *et al.*, 2018) contains 300 images of size 120×120 . These WBC images are collected from the Jiangxi Tecom Science Corporation, China. These images have a yellow background. A Moticam Pro 252A optical

microscope camera with a N800-D motorized auto-focus microscope was used to capture the images. Dataset 2 consists of 100 images of size 300×300. These WBC images are collected from the CellaVision Blog. These images may contain many RBCs around the WBCs which are purple in colour. Figure 11 and Figure 12 shows the sample images from Dataset 1 and Dataset 2 respectively. Figure 13 provides a graphical distribution of the different types of WBCs in each dataset considered.

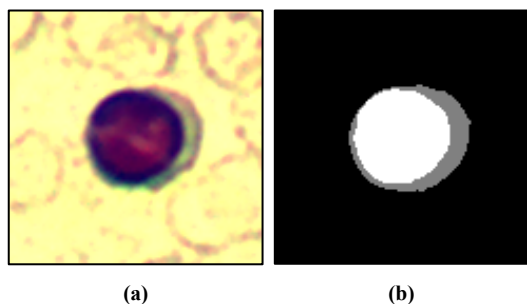


Figure 11. (a) Sample blood smear image from Dataset 1. (b) Ground Truth Image

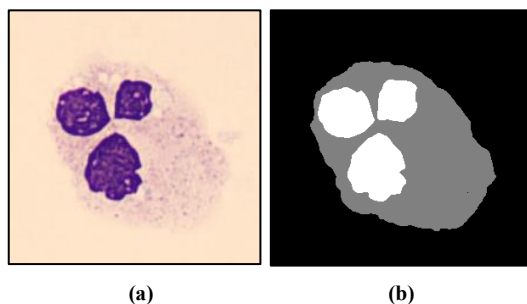


Figure 12. (a) Sample blood smear image from Dataset 2. (b) Ground Truth Image

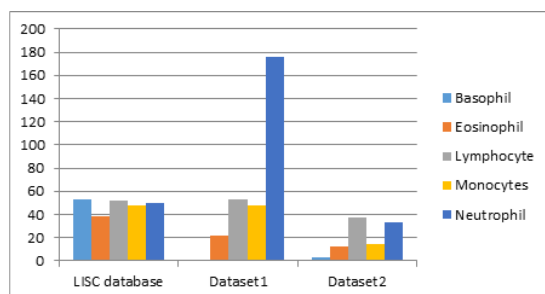


Figure 13. Distribution of WBCs across different datasets

Pre-Processing Techniques

Image Enhancement is one of the crucial steps in Image segmentation. It mainly involves those techniques which improve the visual quality of the images. There are various challenges encountered while segmenting and classifying the WBC Cells. These challenges include noise, occlusion, missing data, etc. The pre-processing technique involves cleaning the data, filling the missing data, resizing the images, orienting, colour corrections, etc. The pre-processing technique highlights the rich hierarchical features that help to detect the region of interest. (Umamaheshwari *et al.*, 2018) put forward an approach to identify Acute Lymphocytic Leukemia (ALL). This was done by separating the WBC cells and classifying them into malignant and benign cells. This method involved pre-processing, segmentation, and extraction of necessary features, and categorizing or classification. In the first step, a microscopic blood smear image was acquired and was resized into 256×256 and converted to grayscale. During the pre-processing stage, grayscale image intensity was enriched by Linear Contrast Enhancement and the image contrast was enhanced using the histogram equalization technique. Finally, the image was brightened except for the nucleus, and the borders were extracted for all the components. The image was filtered using 2-D order statistic minimum filtering. Otsu's Global thresholding mechanism was used for binarizing the image to extract the Region of Interest (ROI) for segmentation. The boundaries were eroded using morphological erosion and tiny holes were filled by the closing operation. Objects were labelled accordingly, and the biggest blob was extracted. Finally, the images were classified using the K-NN algorithm. The test accuracy for classifying the test images into benign and malignant was 95.96%. The overall sensitivity and specificity were 95.92% and 96% respectively.

(Moallem *et al.*, 2017) developed a software to count the infected red blood cells automatically using a smartphone. Machine learning algorithms and image analysis were used in this method. The red blood cells were detected and segmented first. However, due to the presence of WBCs, the process yielded an error since Leukocytes could be misinterpreted as Erythrocytes (Red Blood Cells). As a result of this, leukocyte segmentation was essential. During the pre-processing stage, the ROI was extracted accordingly by the subsampling technique. Hue and saturation information was removed by converting the image to grayscale.

Grayscale-Image was binarized. The binary mask of the subsampled image was obtained using Otsu's method. In the second stage, WBC Cell was detected using Range Filtering. The purpose of using it was to highlight the nucleus edge pixels values. The resultant image was a binary mask with nucleus edge pixels that are assigned a value as 1. These values correspond to partial WBC cells. In the final step WBC cell was segmented by implementing the Chan-Vese level-set method which helped in detecting the Region of Interest, based on curve evolution. The process was terminated when the curve reached the boundary pixel corresponding to a single WBC. This process was repeated for all the WBCs. The dataset used was obtained from Chittagong Medical College & Hospital in Bangladesh. The dataset consists of more than 1300 images. The overall evaluation obtained a Jaccard Similarity Index of 82.28% and a Dice Coefficient of 78.33%.

Rajendran and Kumar (2019) presented an approach using an effective computer-aided technique mainly meant for WBC classification. In this method, it followed the steps like pre-processing, Image segmentation, and classification of images. Haematological images of peripheral blood from a healthy normal individual were used from the LISC Dataset. During the pre-processing stage, techniques such as adjusting the contrast, enhancing the image quality and noise reduction were adopted. In the image enhancement phase the noisy components were removed with the help of different filters such as Gaussian filter along with median filter, and Wiener filter. Otsu binarization technique was utilized to segment WBCs. In the feature extraction process, thirteen features were extracted such as correlation, contrast, energy, mean, etc. To classify the WBCs into 5 different categories, Discrete Wavelet Transform (DWT) was adopted to attain strong properties. The size of the image was reduced with the help of a method known as Principal Component Analysis (PCA). KNN Classifier was used for classifying the WBCs.

Ghane *et al.* (2017) proposed a segmentation technique for segmenting the WBC using a combination of two different algorithms such as K-Means clustering and modified watershed algorithm. To increase the contrast of the images, they proposed a colour adjustment method, based on colour transform. The first step was to convert image from Red, Green, and Blue (RGB) colour space into Cyan, Magenta, Yellow and Black (CMYK) colour space. In the CMYK colour space, it was observed that the contrast of WBCs in the Y component is more than the other components. Consequently, they choose this band for the segmentation process. Next, the WBCs were

enhanced using the linear contrast stretching and histogram equalization of Y image. After enhancing the contrast, a 3×3 minimum filter was applied to decrease spot noise. The minimum filter was repeated three times for getting the best result. The Otsu thresholding method was used to extract WBCs. This technique obtained a similarity score of 92.93%.

Prinyakupt and Pluempitiwiriawej (2015) proposed a segmentation technique which segments and compares the WBC morphology using the linear and naïve Bayes classifiers method. Based on the shapes, sizes, textures, nature of WBC and granules there are 5 different categories of WBCs. Hence, it is a very challenging task to segment the WBCs out of each image. The nucleus present in the WBC appears violet in colour. Among the red, green, and blue colour components, the nucleus region has the least value in the green channel when compared to other regions such as cytoplasm or background. The red and blue channel pixel values were averaged and then the sum was divided by the green channel intensity to enhance the nucleus. The redistribution of the image intensity was done using Histogram Equalization to cover the whole range of intensity in both images and then the image was binarized. In the pre-processing step, the images were subject to erosion operation and then expanded to fill the tiny holes using dilation operation. These operations were applied to the nucleus of the enhanced WBC image to remove any noisy pixels.

Dual-Threshold Method for WBC segmentation from Acute Lymphoblastic Leukemia Images was proposed by (Li *et al.*, 2016). The three phases of the proposed dual-threshold method included pre-processing the image, threshold-based segmentation, and post-processing the segmented image. Two images were obtained in the pre-processing phase for further processing. They were contrast stretched and then transformed into HSV colour space to give the H component. In the threshold segmentation stage, Image backgrounds were extracted, Red Blood Cells were separated accordingly and then the optimal threshold was selected. The single threshold method based on H channel image was utilized for RBC separation and the background was separated using the single-threshold method based on RGB colour space. To find the optimal threshold, a method termed as golden section search was used. During the post-processing, median filtering and mathematical morphology were used for the noise removal.

Computer-aided system for Leukocyte nucleus segmentation was proposed by (Sapna *et al.*, 2020). Classification of leukocytes was done based on the characteristics of the nucleus. RGB colour channels and CIE-LAB colour space were utilized in this

work. To obtain a modified grayscale image, the mathematical operations were performed on the image where the WBC nucleus could be easily differentiated from the other components such as RBC, Platelets etc. The nucleus of a WBC and the WBC cytoplasm are distinguished using the CIE-LAB colour space. The difference in the contrast of the WBC nucleus and WBC cytoplasm was identified by the 'b' and 'a' colour component in $L^*a^*b^*$ colour space. Therefore, it was utilized in generating the image and further morphological opening followed by erosion were performed to enhance the image quality.

Segmentation Techniques

Several types of segmentation technique have been proposed and implemented by the researchers. However, the aim of segmentation process changes depending on the goal of the segmentation task at hand. Whether it be RBC, WBC, or tissues, the methods applied below have been proven to provide efficient results.

A colour-based segmentation technique was implemented by (Mahmood *et al.*, 2013). The CIELAB colour space was formed from the original RGB image. The 'b' channel was used to segment the WBC. Purplish stain skewing to blue colour of the WBC is due to the Wright's stain, which impacts the 'b' channel. Since the RBCs are darker than the background, they were segmented using the 'L' channel. The approximate value of the 'b' channel in WBC is 80 and the RBC has an 'L' value of approximately 130. The effect of the default values on the CIELAB extraction gave the segmented WBC. This method obtained an accuracy of 81%.

Al-Muhairy and Al-Assaf (2005) proposed an automatic WBC segmentation technique using the Watershed algorithm. The distance transform technique was performed on a binary image acquired by the Otsu's Binarization Thresholding technique. This method spots the local minimum. These watershed pixels were considered as the boundary of an individual instance. And then, after being initiated by a watershed algorithm, each region was filled with different colour-components. The nearby valley ran into overlapping as the regions grew. To prevent overflow, an elevated watershed line was created. Each group of nonzero values was detected and differentiated from each region separately. The original images were masked with watershed images to get the actual segmented WBCs. To reduce the background and to better prepare the segmented object for further processing, a bounding box was used. The area of the bounding box was used for the WBC classification. The threshold value was obtained while comparing the WBC with other elements. Aspect Ratio (AR) of the

bounding box and the colour averaging are the two other features that were used for WBC classification. This method obtained an accuracy of 80%.

Ananthi and Balasubramaniam (2016) put forward an approach for the segmentation of the WBC Nucleus by using the idea of fuzzy sets. The uncertainties in terms of brightness values were handled by the fuzzy sets. Initially, to remove the noises, adaptive median filter was applied on the images. Since the 'S' component of the Hue Saturation Value (HSV) colour space identifies the WBC nucleus clearly, it was chosen for the enhancement of the image with the help of gamma equalization. The logarithmic function with the Fuzzy set was considered as a membership function and in terms of non-membership function, a Yager generating function was used to determine the threshold. However, to acquire the threshold, the comparison of the segmented image along with the ideal segmentation result was necessary. The accuracy obtained for the datasets ranged from 79% to 94% with respect to datasets used.

Leukocyte Segmentation based on Colour Segmentation and Active Contour Model. The images are subjected to RGB colour normalization to decrease the luminescence. Highlighted regions and shadows were removed, and this helped in identifying the object. Illumination correction was used to remove the uneven illumination in the images. Colour transformations and Gaussian filters were mainly applied to remove the noises and to enhance the image quality. And an active contour model was used for the segmentation. The active contour method needs an initial contour. It divided an image into two regions i.e., foreground and background using a level set function. This method obtained a sensitivity of 90.13%.

PuttamadeGowda and Prasanna (2019) proposed a method of WBC Segmentation Using Integrated Process of Improved Spectral Angle Mapper, Orthogonalization with K-Means Clustering, Gram-Schmidt. The RGB images were converted to grayscale and the median filter was used to remove the noises. Spectral angle was calculated between the test and reference spectrum using Improved Spectral Angle Mapper (ISAM) method following which a Gram-Schmidt orthogonalization procedure-based orthonormal vector was applied to the above images. The accurate segmentation of WBC cytoplasm and nucleus was achieved by applying K-means clustering on these orthonormal images. This method obtained an accuracy of 98.38%.

Ravikumar (2016) proposed Image segmentation and classification of WBC using fast Relevance Vector Machine (RVM) and extreme

machine learning. The segmentation of WBCs was done using Fast RVM models. Compared to the standard Relevance Vector Machine-based classification the Fast Relevance Vector Machine model has much faster testing time. A small set of training data was used to evaluate the decision function. 1D histogram obtained by Fast Relevance Vector Machine was used for obtaining the Relevance Vectors (RVs). The WBCs were then segmented from the original images. Efficiency was calculated using precision and recall. The results obtained were in the range of 65% to 90% for all three methods involved.

Cao *et al.* (2018) proposed a novel algorithm for the segmentation of White Blood Cells in peripheral blood smear image samples. HSG transformed image made up of Hue, Saturation and Green color was constructed and the thresholds for segmentation were calculated using Stepwise Averaging method. Finally, the nucleus was segmented using the average of threshold values of cytoplasm and nucleus. The average segmentation accuracy was 93.75%.

Al-Hafiz *et al.* (2018) proposed an automatic and effective segmentation technique for RBCs. The approach makes use of tuned threshold values and performs cell segmentation using the Canny edge detection method. A pre-filtering process was performed before the segmentation task. The boundary-based methods and morphological operators were used for RBC segmentation in biomedical images followed by threshold computation. This method gave an accuracy of 87.5% for detecting the RBCs. Overall, the method provided higher average accuracy and average similarity.

Kowal *et al.* (2020) concentrate on cytological examinations for Morphometric nuclei analysis. It implemented an approach that is a combination of watershed and CNN to segment the WBCs nuclei present in the breast cancer cell images. Cellular objects were highlighted with the help of a pre-processing technique, the colour deconvolution method, followed by CNN for segmentation. Overlapping nuclei were separated using a seeded watershed algorithm. The degree of matching was calculated using Jaccard and Hausdorff distance. The distance between discovered objects and reference nuclei was found. It exceeded Otsu's thresholding and adaptive thresholding. The maximum Jaccard coefficient values recorded for CNN were 93.3% for benign and 90.5% for malignant.

Naylor *et al.* (2017) implemented an approach to analyse and interpret stained tumour cells in cancer diagnosis. It aimed to develop a way to analyse large datasets of complex images to draw

conclusions and to study tissular and cellular phenotypes. The main concern in these methods is being able to achieve automatic segmentation of cell nuclei. The authors presented a fully automated deep neural network that performs segmentation tasks based on the histopathology image data, trained, and processed using posterior probability maps. This helped in segmenting cell clusters efficiently. The dataset has been specifically generated for this study.

Bidart *et al.* (2017) provide a solution for post-NAT (Neoadjuvant Therapy) image processing. They developed an automatic method that localized and classified nuclei among Lymphocyte, benign epithelial, and malignant epithelial figures. The samples were taken from slides containing breast cancer tissues. FCN was developed to satisfy both the tasks at hand. The methodology consisted of three main steps. Training FCN, pre-trained FCN applied to image patches, Localization - resulting in generation of four heatmaps followed by a non-maximum suppression algorithm for location estimation and classification. The classification accuracy obtained was 95.2%.

Adollah *et al.* (2008) reviewed and summarized few of the common methods of segmentations that are being used in biomedical image processing of blood smear images. The whole image is divided into unique disjoint regions from which features are extracted. The main goal of any segmentation task is retaining maximum useful information about the objects under consideration. There is no single best technique to solve an image segmentation problem in a specific problem domain. There are several instances where multiple techniques have been combined to provide an efficient solution. The effort is made to find an affordable and efficient method.

Ronneberger *et al.* (2015) discussed the advantages of U-net in biomedical image segmentation. The network was made up of contraction path that broke down the image to a low-resolution feature map and a corresponding expansion path that provided localization. The advantage of this network was that it could be trained lengthwise from a few images and relied on data augmentation to use the sample efficiently. This network achieved excellent performance for complex segmentation tasks. The best average IOU recorded was 92.03% for the data set PhC-U373.

(Kutlu *et al.*, 2020) concentrated on the study of deep neural networks to improve the efficiency of existing blood test devices. It used R-CNN to identify and classify the blood cells to their respective types. The model was tested on BCCD and LISC datasets. The reason for using R-CNN was, it helped to identify different types of WBC's

present in a single image. The base architecture used for training was CNN. Different CNN architectures were tested with full learning and transfer learning to ensure automatic detection of WBC species. ResNet50 architecture showed the best performance and cells were identified with an overall accuracy of 97%.

(Zeng *et al.*, 2019) implemented a U-net based network for the segmentation of the nucleus that stands for Residual Inception Channel Attention U-net. It made use of the concepts of residual block, multi-scale, and channel attention mechanism to provide more accurate results. The authors also made comparisons with two traditional and two original CNN methods and with the original U-net model. The methods were evaluated based on the Jaccard Index, F1-score, and Dice.

Grimmeiss *et al.* (2019) made use of machine learning to develop a model derived from U-net for WBC segmentation which made use of Adam optimizer. It produces binary results (consists of cell and background) and multiclass results (consists of cytoplasm, nuclei, and background). Binary results had an Intersection over Union (IoU) value of 93.9% and multi-class results had an accuracy of 94.5% and 82.8% for the nucleus and cytoplasm respectively. The major challenge faced was concerning cell clusters.

Roy and Sasi (2018) proposed a framework that had two-phase classification. In the first phase, the images of blood smear data were classified into classes of WBCs using a Support Vector Machine having four hyperplanes. In the second phase, CNN technique was used for classification. The CNN model had receptive fields where neurons only connected to a small region which is present in the layer below it. Each filter had the same weights and was fully connected. Finally, a sigmoid activation function was applied.

Weng *et al.* (2019) extended Neural Architecture Search (NAS) for segmentation of medical images. For the segmentation of cells, U-net Architecture was used. The planning was stacked by Down-Sampling and Up-Sampling the Cells.

Tobias *et al.* (2020) utilized the Deep Learning technique called the Faster R-CNN model to classify variants of WBCs and RBCs. Eosinophils achieved the highest testing accuracy of 99%.

Sharif *et al.* (2020) proposed a method based on YOLOv2 for WBC localization. DarkNet-19 was

used as the network base for the YOLOv2 model. Feature extraction was done using LeakyReLU-18 of DarkNet-19. Thus, the proposed method localized the WBCs among non-blast and blast cells. Using Particle Swarm Optimization (PSO), optimized features were extracted. The obtained Feature vector was fed to a series of classifiers i.e., optimized discriminant analysis (O-DA) & optimized naïve Bayes (O-NB). Experiments were conducted on ALL-IDB2, ALL-IDB1, and LISC datasets. For O-DA classifiers on the LISC dataset, accuracy obtained was 99.5%.

Sapna *et al.* (2020) proposed a computer-aided system for Leukocyte nucleus segmentation. While converting blood smear image to grayscale, colour information is usually lost. To prevent this, pre-processing method was used. To segment the WBCs, Fuzzy C-Means clustering technique was implemented. Back propagation algorithm with Neural Network and SVM Classifier was used for classification. Performance of proposed method were compared, and it was found that Neural Network Classifier was better for WBCs classification based on the extracted nucleus characteristics. It achieved a classification accuracy of 92.8%.

Hegde *et al.* (2018) proposed Tissue Quant method to enhance the image to address variations of illumination in the images and to manage colour shade variations in the image. Dice similarity coefficient obtained for detecting nuclei was 0.95. For the classification of WBCs texture and shape features were used. The accuracy and sensitivity obtained was 99% for WBC classification.

Hegde *et al.* (2019a) implemented an approach to enhance image by minimize the brightness variation and colour variation present in the images. To detect the approximate region of the nuclei, Tissue Quant method was used. By considering images which are present in the ALL-IDB2 dataset, robustness of the proposed method was evaluated. The Dice Coefficient of the proposed method was around 0.97.

Branislav *et al.* (2018) proposed two different neural networks, U-Net and PSPNet for WBC segmentation. Simultaneously, the cytoplasm and nucleus were segmented. The overall IoU obtained for segmentation was 0.9. Table 1 provides a detailed overview of different techniques proposed for WBC segmentation in the literature.

Table 1. Overview of Segmentation Techniques

Authors Reference	Techniques used	Dataset Used	Performance	Remarks
(Mahmood <i>et al.</i> , 2013)	CIELAB colour space transformation, 'b' channel used to segment WBC	108 images Acute Lymphoblastic Leukemia Image Database	~80%	Cell-counting, segmentation, and identification of RBC's
(Al-Muhairy and Al-Assaf, 2005)	Otsu's thresholding & watershed algorithm	Neut – 120 Eosi – 67 Lymph – 96 Mono - 76	Neut – 90% Eosi – 45% Lymph – 100% Mono – 79% Overall = 80%	4 types of WBCs were considered except basophils. The combination of these techniques resulted in adequate segmentation.
(Ananthi and Balasubramaniam, 2016)	Fuzzy sets and HIS colour transformation	CellaVision - 540 images	~90%	Interval-valued intuitionistic fuzzy set generation by minimizing the ultra-fuzziness.
(Angkoso <i>et al.</i> , 2018)	RGB colour normalization, illumination corrections & active contour model for segmentation	352 images Mononuclear – 57 Polynuclear - 295	90.13%	Divides the images into foreground and background for ease of extraction
(PuttamaGowda and Prasanna, 2019)	Improved Spectral Angle Mapper, Orthogonalization using Gram Schmidt k-Means clustering for WBC cell extraction	All 5 types	98.38%	Accurately extracts cytoplasm and nucleus of white blood cells
(Ravikumar, 2016)	WBC segmentation using Fast-RVM, subset features extracted using RVM	All 5 types	~90%	Preserved the image characteristics and effectively reduced the computational time
(Cao <i>et al.</i> , 2018)	HSG transformed images segmented using SWAM method	Set 1 -70853 Set 2 -6331	93.75%	Minimizing fuzzy divergence was used for cytoplasm segmentation, performed better than interval-valued intuitionistic fuzzy sets
(Al-Hafiz <i>et al.</i> , 2018)	Canny edge detection method, boundary-based methods, and morphological operators	5 image sin DIC format	87.5%	This method provides higher average accuracy and average similarity
(Kowal <i>et al.</i> , 2020)	Watershed algorithm combined with CNN to segment nuclei in breast cancer images	80 images Malignant – 10 Benign - 10	Benign – 93.3% Malignant – 90.5% Overall = 81%	Pre-segments the nuclei and deals with overlapping cells
(Naylor <i>et al.</i> , 2017)	Fully automated deep neural networks for segmentation, trained using posterior probability maps	33 images 2754 annotated cells	PangNet – 92.4% DeconvNet – 95.4% FCN – 94.4% Ensemble – 94.4%	Analyses large datasets of complex images, studies tissue, and cell phenotypes.
(Bidart <i>et al.</i> , 2017)	Pre-trained FCN	27000 annotated nuclei	95.2%	Segmentation of nuclei in histopathology images.
(Ronneberger <i>et al.</i> , 2015)	U-Net segmentation	30 images Drosophila first intra larva ventral nerve cord	92.03%	Excellent performance for complex tasks. Relies on augmentation for more efficiency
(Kutlu <i>et al.</i> , 2020)	R-CNN	LISC - 250 BCCD - 364	97%	Multiple CNN architectures are tested using full learning and transfer learning for best results
(Zeng <i>et al.</i> , 2019)	RIC-UNet	TCGA – 53662	Simple – 89.68% Complex – 82.80%	Mechanism to automatically select features from images of different resolution.
(Graham <i>et al.</i> , 2019)	U-Net with Adam optimizer	Dataset 1 – 64x64 Dataset 2 – varying. Dataset 3 – 480x480	Nucleus - 94.5% Cytoplasm - 82.8%	Both binary and multiclass results comparison
(Weng <i>et al.</i> , 2019)	Extended NAS	MRI – 1250 CT – 2847 Nerve - 5635	97.37% 97.4% 88.1%	Provides better performance with few parameters
(Sharif <i>et al.</i> , 2020)	DarkNet-19 based YOLOv2 model	ALL-IDB1- 260 ALL_IDB2- 108 LISC - 242	99.5%	Localizes the WBCs among non-blast and blast cells, uses PSO for optimized feature extraction
(Sapna <i>et al.</i> , 2020)	Fuzzy C-means clustering	LISC - 242 MISP - 150	92.8%	Neural network better classified the nucleus characteristics, count of WBC's.
(Hegde <i>et al.</i> , 2018)	Enhances nucleus in the images on illumination variations, Tissue Quant tool is used.	117 images	Baso – 100% Lymph-100% Overall = 95%	Manages colour shade variations in the image.
(Hegde <i>et al.</i> , 2019a)	Colour segmentation	ALL-IDB2 - 260	97%	Manages brightness variations
(Hegde <i>et al.</i> , 2019b)	Colour transformation with tissue quant and brightness variation method	ALL-IDB2 –160 Dataset 2 – 160	Dataset 1 - 97% Dataset 2 – 96%	Large number of labelled data is required with good infrastructure.
(Branislav <i>et al.</i> , 2018)	Unet and PSPNet for WBC segmentation	Dataset 1 300 images Dataset 2 100 images	PSPNet - 97% UNet – 96%	Both nucleus and cytoplasm are extracted simultaneously; segmentation is almost identical, with smoother borders.

Classification Techniques

Leukocytes are of five different types. Those are - neutrophils, eosinophils, lymphocytes, monocytes, and basophils. Each type of WBC has different characteristics and properties. So, identifying the WBC and its type is important. The correct identification and counting of the different WBCs are crucial steps. Several automatic classification methods have been implemented to achieve fast and accurate classification.

Hegde *et al.* (2019b) proposed an approach for classification of WBCs using traditional neural network and deep learning approach. Both the methods performed well with overall accuracy and sensitivity of over 99%.

Wijesinghe *et al.* (2020) attempted to classify WBCs using a VGG-16 classifier. Out of the 4100 raw microscopic images considered initially, which were obtained from 40 different canine blood smears, size of the dataset was increased by augmenting the images using rotation and thereby reached 12,500 images samples. The augmented dataset included 2,500 images of each class. The overall accuracy obtained for the train set and test set were 99.93% and 95.89% respectively.

Macawile *et al.* (2018) utilized the saturation component of HSV with Binary Large Object analysis for leukocytes segmentation and three Convolutional Neural Network model namely AlexNet, GoogLeNet, ResNet-101 along with transfer learning were used for classifying the WBCs. The generated overall sensitivity of the model was 89.18%, specificity of 97.85% and accuracy of 96.63% was obtained.

Hegde *et al.* (2019c) implemented an image processing approach at its base and CNN approach for feature extraction. In total 113 features were extracted. The shape, colour and texture features were extracted using traditional image processing. WBCs were identified by performing two steps. In the first step of this identification process, WBCs were classified into normal and abnormal cells. In the second step the normal WBCs were classified into its five types. Nearest Neighbour classifier was used for the normal WBC classification. The Local Binary Pattern (LBP) representation was applied for grayscale images to extract texture features as it emphasises the local textures. The accuracy obtained for the first step was 99.7% and average accuracy of 98.4% was achieved for WBC classification step.

Togacar *et al.* (2019) proposed an approach for feature extraction using AlexNet, LeNet, and VGG-16 architectures which also performed the classification. The AlexNet architecture was used for better classification and attribution. This architecture consists of the convolutional layer, pool

layer, and fully connected layer. The convolutional layer convolves a particular filter over the entire image. Filters can be of different sizes, such as 3×3 or 5×5 elements. The convolution processes were added to the images from the previous layer to form the filter output. The convolution process resulted in an activation map. The pooling layer used in the AlexNet architecture is used to reduce image size by preserving attributes. Its classification results included Decision Tree, Quadratic discriminant analysis, Linear Discriminant Analysis, Support Vector Machine, KNN. All the mentioned classifiers were compared with Softmax classifiers. An accuracy of 97.78% was achieved for the QDA.

Yildirim *et al.* (2019) discussed the Classification of Leukocytes using Deep Learning Method. This method was developed to diagnose diseases based on cellular properties. The majority WBCs - Eosinophil, Lymphocyte, Monocyte and Neutrophil were classified. MATLAB application environment was used for the classification. AlexNet, Resnet50, Densenet201 and GoogLeNet Architecture were implemented. This was followed by Gaussian and median filters which were applied separately to each image. The new images obtained were classified again using the same architectures as before. The obtained accuracy percentages after applying Gaussian filter were 82.16%, 80.02%, 83.44% and 75.21% for previously listed architecture respectively.

Sahlol *et al.* (2020) worked on Leukemia cell identification. The proposed method used Improved Swarm Optimization of Deep Features. This improved the efficiency of Leukemia cell Classification. A classification approach that combined two networks was developed and WBC Leukemia image classification was proposed in this work. Initially it used a deep convolutional neural network (VGGNet) for feature extraction purposes from WBC images. It was then followed by a statistically enhanced Salp Swarm Algorithm (SESSA) to filter the resulting features. It was used to extract only relevant features and eliminated unnecessary ones. The SESSA optimization provided feature reduction by narrowing down the features number from 25K to 1K. With this came the advantage of improvement in performance. SVM classifier was used for the classification. This achieved an overall accuracy of 87.9%.

Su *et al.* (2014) developed an approach based on neural networks to classify WBCs. The segmentation algorithm was concentrated on finding a region of discrimination in the white blood cell tones in the colour space. Then they divided the 20 features into geometrical, colour, and LDP features. Lastly, three different neural-network-based classifiers were used. The Multilayer

Perceptron (MLP), Hyperrectangular Composite Neural Networks (HRCNNs) and Support Vector Machine (SVM) were adopted for classifying white blood cells into one of the five types. Out of the three techniques, MLP provided the highest performance with accuracy percentage of 99.11% (Abdullah *et al.*, 2019) proposed WBC classification using machine learning. 35 different parameters in a feature vector were used for forming the statistical and geometric features. It was used as the input parameter for 6 different machine learning algorithms for classification. The classifiers used were Naive Bayes, KNN, and random forest, SVM, Decision Tree and Multinomial Logistic Regression (MLR). The MLR classifier obtained the best performance with an accuracy of 95%.

Zhao *et al.* (2017) discussed an automatic system to detect and classify leukocytes based on CNN. It utilized the relation of colour channels R and B. With this they generated an R-B image and applied the morphological operation to remove distortions and complete the nucleus. These operations were followed by an algorithm which merged the lobes of the nucleus to help in better detection of WBCs from peripheral blood images. Eosinophils and basophils were first distinguished from the remaining three types of WBCs using PRiCoLBP features and SVM. Later CNN was used to extract features for the remaining three kinds of WBCs, and a random forest was used for distinguishing them. This method achieved overall 92.8% accuracy for WBCs classification.

Table 2 provides a detailed overview of different kinds of state-of-art classification techniques for WBC identification and classification.

Counting the number of WBCs

The most critical measurement parameter in WBC diagnosis is the WBC count. This information is effectively used for evaluation of cancer therapy and to diagnose various hidden infections. Traditional practices involved manual WBC counting which is laborious and time consuming. A very subjective assessment of these factors led to the development of a computer-aided system (CAS) implementing a robust solution.

Safuan *et al.* (2017) proposed a technique in which they counted WBCs in blood smears. This paper discussed the various strategies for segmentation. To circle the object of interest, Circle Hough Transform (CHT) was used. This was achieved by using an overlapped region which

highlights WBC regions in the image. Thus, the count of WBC was obtained. The approach reached an accuracy of 97.79%.

Iterative Structured Circle Detection Algorithm was implemented by (Alomari *et al.*, 2014). The proposed method used automatic segmentation and counting of red and white blood cells. This algorithm had undergone several modifications including an initialization step to find 8-neighbor connected component. Along with counting, the proposed model features enhanced the probability of selecting the correct circle from four candidate circles. It also detected abnormal cells, implemented iterations of a dynamic number, and improved detection of overlapping cells. The F-Measure result was 93.9% for WBC and 96.4% for RBC counting.

Mahanta *et al.* (2019) proposed an approach to detect and count cells. Images were taken from 12 different blood sample slides that made up to 270 microscopic blood smear images. A total of 249 images were used in the experiment. The images were pre-processed using a gaussian filter followed by LAB colour space transformation. Later they were transformed to binary based on a certain threshold value and morphological operations were performed on it. Convex hull was applied to the resultant image. Finally, the cells were counted using the contour detection function in open-cv. The statistical accuracy was tested using Karl-Pearson's t-test. The accuracy obtained was 95.59% for platelets and 100% for WBCs respectively.

Dridi *et al.* (2018) conducted a thorough study of malarial infection in RBCs because of its difficulty in dealing with an overlap of cells and low resolution of images. This approach overcomes the obstacles by using Otsu's thresholding method during pre-processing and two masks during segmentation by performing the morphological operations. It was seen that the accuracy of the identification was very high. It overcame many challenges like differences in colour and illumination too. To calculate the infected cells in the image, custom masks were created for each of the regions. The accuracy obtained was 98.59%.

Sharma and Kinra, (2014) proposed an approach to detect and count the WBC in sample blood images. K-means clustering was used for colour-based clumping and histogram method was applied to the pre-processed images. The pre-processing step was done to analyse the coloured pixels count and their intensities. Finally, cell counting was performed by identification of the affected area containing WBCs.

Table 2. Overview of Classification Techniques

Authors Reference	Classifier/Features Used	Dataset Used	Identified Classes	Accuracy (%)	Remarks
(Wijesinghe <i>et al.</i> , 2020)	VGG16	4100 images 135 Basophils 319 Eosinophils 773 Lymphocytes 552 Monocytes 2483 Neutrophils	Basophil	92.31	This algorithm can be integrated with raw microscopic images obtained from an automated microscope for the WBC differential counting
			Eosinophil	93.36	
			Neutrophil	99.59	
			Lymphocyte	98.70	
			Monocyte	95.50	
				Overall = 95.89	
(Macawile <i>et al.</i> , 2018)	AlexNet GoogLeNet ResNet-101	ALL-IDB dataset	Basophil	99.81	CNN was used for classifying all types of WBCs. AlexNet gave better result of classification.
			Eosinophil	99.44	
			Neutrophil	95.88	
			Lymphocyte	95.51	
			Monocyte	95.5	
				Overall = 96.63	
(Hegde <i>et al.</i> , 2019c)	Nearest Neighbour classifier	280 images 32 Basophils 31 Eosinophils 26 Lymphocytes 28 Monocytes 67 Neutrophils 136 abnormal WBCs	Basophil Eosinophil Neutrophil Lymphocyte Monocyte	98.4	WBCs were identified by performing two steps. In the first step the WBCs were classified into normal and abnormal cells. The second step classified the normal WBCs into its five types.
(Togacar <i>et al.</i> , 2018)	Feature extraction using AlexNet, LeNet and VGG-16 Classification Using AlexNet	12,435 images. 3120 Eosinophils 3102 Lymphocyte 3091 Monocytes 3122 Neutrophils	Eosinophil Neutrophil Lymphocyte Monocyte	97.78	Classification results included Decision Tree, Quadratic discriminant analysis, LDA, Support Vector Machine, KNN. It classified 4 types of WBCs
(Yildirim <i>et al.</i> , 2019)	Alexnet, Resnet50, Densenet201 and GoogleNet	9663 images taken from Kaggle database	Eosinophil Lymphocyte Monocyte Neutrophil	80	Gaussian and median filters were applied to the database images. The same architecture was used to re-classify the new images. Applying Gaussian and Median filters to the images gave better results.
(Sahlol <i>et al.</i> , 2020)	VGGNet Statistically Enhanced Salp Swarm Algorithm (SESSA)	ALL-IDB2 dataset	Basophil Eosinophil Neutrophil Lymphocyte Monocyte	87.9	No pre-processing or manual steps are required. This method works fully automated on the raw images
(Su <i>et al.</i> , 2014)	MLP, HRCNN and SVM	CellaVision Competency Software Databases contains slides stained with either a MGG or a Wright staining	Basophil Eosinophil Neutrophil Lymphocyte Monocyte	99.11	MLP gave the highest performance for classification.
(Abdullah and Turan, 2019)	Naive Bayes, KNN, Random Forest, SVM, Decision Tree and MLR	350 images	Basophil Eosinophil Neutrophil Lymphocyte Monocyte	95	The MLR classifier obtained the best performance.
(Zhao <i>et al.</i> , 2017)	PRICoLBP, SVM, CNN and Random Forest method	CellaVision database ALL-IDB dataset	Basophil Eosinophil Lymphocyte Monocyte Neutrophil	100	This method first distinguished the eosinophils and basophils from the remaining three types of WBCs. CNN was used to extract features of the remaining 3 types of WBCs and Random forest algorithm was used for classifying them.
				70	
				74.8	
				85.3	
				97.1	
				Overall = 92.8	

Conclusion

In this paper, different works in the domain of peripheral blood smear analysis used for diagnostic purposes are summarized. Because of difficulties in manual identification and classification of WBCs, the computer-aided technology adopted to extract and classify the WBCs reduces time and errors. We have carried out a survey on some of the popular segmentation and classification techniques used by other researchers.

The usefulness of WBC Segmentation and Classification can be realized in specifying if a smear represents a normal case or not. It can be used as a screening tool to reduce the workload on pathologists. With the use of an automated method, we could obtain robustness for handling illumination, colour, and texture variations in peripheral blood smear images. Hence a system that could collect information about all the irregular regions in all views for a given specimen would become handy. We could shift the dependency on manual evaluation for the regions on to an automated system thus making it more helpful for applications in telemedicine.

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