Towards Prediction of Platelet Count and Classification of White Blood Cell Type, State and AML Stage from Blood Smears

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ABSTRACT: Blood smear analysis plays an important role in the diagnosis of diseases. It is performed by doctors through visual examination of blood smears under microscope. But this is time consuming, tedious and susceptible to error. Hence automated analysis of blood smears is essential. The objective of the paper is fourfold: To determine (i) platelet count which could be used as a preliminary screening for dengue (ii) the type and count of White Blood Cells(WBCs) (iii) the normal and abnormal WBCs and (iv) the stage of Acute Myeloid Leukemia (AML) if abnormal from blood smear. To determine the platelet count, mapping and gray level transformation are done prior to edge detection and morphology based segmentation. To determine the type and count of WBC, the stage of AML and to determine whether WBC is normal or abnormal, threshold based segmentation is done followed by morphological operations to extract the structure of the nucleus within the blood smear. Features are extracted and further fuzzy classification is done. The experiment is conducted with real data set comprising of blood smear images from Tirunelveli Medical College Hospital (TVMCH). Besides these, images available in ASH Image Bank are also used. The results guarantee an accuracy of 94% for WBC type and Acute Myeloid Leukemia (AML) stage classification. The results are validated with the results of pathologist.

Keywords: White Blood Cells (Wbc), Platelets, Threshold Based Segmentation, Morphological Operations, Fuzzy Classification, Feature Extraction

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1. Introduction

Blood smear is composed of red blood cells (rbc), white blood cells (wbc), platelets and plasma. Blood smear analysis is done by counting cells from blood microscopic images.wbc consists of nucleus and cytoplasm and it is categorized into five types namely, monocyte, lymphocyte, neutrophil, basophil and eosinophil. The color, texture, size, shape and morphology of the nucleus and cytoplasm differentiate these cell types [1]. Each one of them plays an important role to ensure healthiness. The wbc may be normal or abnormal. Lack or excessive amount of wbc is termed abnormal and will cause leukemia. Leukemia cells are referred as blast cells. There are two types of acute leukemia namely acute lymphoblastic leukemia (all) and acute myeloid leukemia (aml). This paper focuses on aml stage classification. There are two classification of aml describes 8 main types, m0, m1, m2, m3, m4, m5, m6 and m7. Of these m0 to m3 are related to wbc and m4 to m7 are related to red blood cells (rbc). Among them only m1 and m3 are taken for this study considering data availability as only these two types of AMLs are predominant in

southern Tamil Nadu.

Platelets are element of blood whose function is to stop bleeding. The platelets are biconcave discoid structures shaped like lens and are of 2-4 ¼m diameter. The normal platelet count ranges from 1, 50,000 to 4, 50,000 platelets per microlitre of blood. Having more than 4, 50,000 platelets is a condition termed as thrombocytosis. A count of less than 1, 50,000 platelets is known as thrombocytopenia. The platelet count is obtained through a routine blood test called a Complete Blood Count (CBC) [2]. Platelet count is done manually using a hemocytometer or with an automated analyzer.

Currently all the said objectives are met out manually and take up to five days. Besides this, blood smear analysis can be made free of cost. Many automated systems are available in specialized hospitals where the blood samples are treated chemically using a lyze which is imported and hence it makes analysis costly. As a result there is a strong need to automate blood smear analysis so as to reduce the error rate as compared to manual estimation and to reduce the cost associated with automated analyzers. Also, Blood related diseases have caused serious illness leading to death. To reduce the death rate, early detection of diseases must be done. Simple and easy methods need to be identified to help clinician during blood analysis. One of the fields that can be used to address these issues is image processing.

Hence, the objective of this paper is to automate the following: (i) determination of platelet count (ii) determination of the count and classification of type of WBC, (iii) classification of whether the WBCs in the Blood Smear are normal or abnormal and (iv) if abnormal, classification of what stage of Acute Myeloid Leukemia (AML) it is. The rest of the paper is organized as follows: Section 2 discusses related works. Section 3 discusses the proposed work. Experimental Settings and analysis of the proposed work are given in Section 4. Performance comparisons are discussed in Section 5. Conclusion and Future Scope are drawn in Section 6.

2. Related Works

Several works have been reported for WBC segmentation from blood smears [3, 4, 5, 6, 7, 8, and 9]. T. Markiewicz et al. [10] have developed a system to select the diagnostic structures for feature extraction of the morphologically preprocessed images and have reported an accuracy of 87%. H. Ramoser, et al. [11] have presented a method for automated segmentation and cell classification based on a set of features and using Support Vector Machine (SVM) for classification. They have reported an accuracy of 95% for segmentation and 75% to 99% correct classification with reject option. Mostafa Mohamed A. Mohamed et al. [12] have developed a technique for automatic WBC nuclei segmentation relying on enhancing the color of the nucleus and filtering the image. Small objects are eliminated using morphological operations. The method has reported an accuracy of 85.4%. The segmentation accuracy of eosinophil, lymphocyte, basophil, are reported to be 90.1%, 78.3% and 78.6% respectively. Anjali Gautam et al. [13] have developed a method for the classification of WBC based on morphological features. Results indicate that lymphocytes are classified with an accuracy of 100%, and monocytes with 50%, and the overall accuracy of the method is reported as 73%. Subrajeet Mohapatra et al. [14] have proposed a method for automated leukemia detection using Hausdorff Dimensions (HD) and Contour Signature (CS) in blood microscopic images. Shape, color, and texture features are also considered. The accuracy of their method is reported as 95%. Razali Tomari et al. [15] have developed a system for RBC classification by employing Otsu segmentation on green color channel image with a sense of post processing filter. Compactness and HU moment features are used to distinguish the normal and abnormal shape. ANN is used for classification and accuracy is reported as 83%. J. E. Arco et al. [16] have developed a method to automate enumeration of malaria parasites from blood smears using morphological operations. Histogram equalization, morphology or thresholding are employed to estimate the number of malaria parasites in the blood films.

With the available literature no work is reported so far for platelet count estimation and AML stage determination. Much of the works have been reported for WBC segmentation and only one work is reported for WBC classification however, with a 113 minimum accuracy. Most of the works have been attempted for the classification of normal and abnormal blood smears. Hence this work is attempted towards the development of an automated system to meet the objectives.

3. Methodology

In the following sub sections, the methods employed for counting the number of platelets, WBC type classification, classification of normal/abnormal WBC in blood smear and AML stage classification are discussed. Figure 1 shows the overall architecture of the proposed work.



Figure 1. Architecture of the proposed work

3.1 Platelet Count Determination

From the blood smears only the platelets are separated using morphological characteristics. The following are the steps involved:

3.1.1 Mapping Intensity values

Intensity adjustment is a technique for mapping an image's intensity values to a new range. To enhance the contrast of the image and detect platelets more effectively, the intensity values are mapped from original color domain to the domain in which platelets intensity values lie. The intensity is mapped as shown in Equation (1):

$$s = c \log \left(1 + r\right) \tag{1}$$

where, c is a constant and r is intensity of the image.

3.1.2 Gray Scale Transformation

After using the color information of the pixels and enhancing the contrast of the image in the previous step, the image is transformed as a grayscale image of 8 bit depth by eliminating the hue and saturation information while retaining the luminance. The formula for luminosity is given by Equation (2):

$$Lu\min osity = 0.221R + 0.72G + 0.07B \tag{2}$$

3.1.3 Edge Detection

Sobel edge detection operator is used. The Sobel operator is based on convolving the image with a small, separable, and integer valued filter in horizontal and vertical direction and is therefore relatively inexpensive in terms of computations. The operator uses two 3×3 kernels which are to calculate approximations of the derivatives one for vertical. This is given in Equation (3):

$$N(x, y) = Sum \ of \{K(i, j) . P(x-i, y-j)\}$$
 ... (3)

where N(x, y) represents the new matrix resulted after applying the convolution of K to P, the pixel matrix.

3.1.4 Morphology Based Segmentation

Morphological operator always takes a binary image and a structuring element as input and combines them using a set operator. It processes objects in the input image based on characteristic of its shape which is encoded in the structuring element [1]. The original image and the platelets extracted using morphology based segmentation and are shown in Figure 2.

3.1.5 Platelet Count Estimation and Fuzzy Classification

Once the regions corresponding to platelets are identified in the given blood smear, the total number of platelets must be estimated. This is done by labeling all 8-connected components and counting distinct entities. This gives the platelet count for one image. This count is multiplied by 15,000 to equal the approximate platelet count/ $\frac{1}{4}$ L. Normal range of platelets is 1, 50,000 – 4, 50,000. If the range falls below 1, 50,000 it is said to be thrombocytopenia and if it exceeds above 4, 50,000 it is said to be thrombocytopenia. In case of thrombocytopenia it can be used as a preliminary screening system for dengue fever.

3.2 WBC Type Classification

WBC extraction and counting involves several processes namely gray scale conversion, contrast adjustment, and frequency filtering technique for image enhancement. Meanwhile, for image segmentation, it uses morphological operation and boundaries detection in order to identify the number of objects in the image before counting. Shape and texture features such as area, solidity and energy are used to classify the WBC type.



Figure 2. (a) Original Blood Smear, (b) Platelets extracted

3.2.1 Image Enhancement

Contrast adjustment is used to adjust the intensity values of an image. The concept is similar to threshold operation. In this paper, the spatial filtering used is median filtering technique. Median filtering is particularly effective in the presence of salt and pepper noise. Sharpening an image means to make the image information more sharp or clear with details. Meanwhile, blurring an image is to remove noise.

3.2.2 Segmentation

Morphological operation such as opening, closing, erosion and dilation help to get the original size and shape of the Region of Interest (ROI). At the same time, they improve the accuracy of cell counting by covering holes area in WBC structure and thereby help in accurate boundaries detection [9, 16, 17]. The structuring element is created. Erosion is used to shrink or reduce the size of an image according to the shape and size of the structuring element, while preserving the image information. Dilation is used for increasing the size of object in an image by filling or expanding an image according to shape and size of the structuring element. The output of segmentation after dilation, erosion, opening and closing respectively are shown in Figure 3.



Figure 3. Segmentation output after (a) dilation (b) erosion (c) opening (d) closing

3.2.3 WBC counting and Fuzzy classification

Object counting is one of the popular techniques used in image processing and analysis to count the number of objects in images. Bounding box technique is used to automatically crop the objects in image. Feature selection greatly influences the classifier performance; therefore, a correct choice of features is a very crucial step. In order to construct an effective feature set, several published articles are studied, and their feature selection methodology is observed. It is noted that shape and GLCM features have been widely used for classification [9, 10]. The shape features are as follows:

(i) Area

It is determined by counting the total number of non zero pixels within the image region.

(ii) Solidity

The ratio of actual area and convex hull area is known as solidity and is also an essential feature for blast cell classification. This measure is defined as in Equation (4):

Solidity =
$$\frac{Perimeter^2}{Convex hull Area} ...(4)$$

(iii) Perimeter

It is measured by calculating distance between successive boundary pixels.

(iv) Compactness

Compactness or roundedness is the measure of nucleus. It is given by Equation (5):

$$Compactness = \frac{Perimeter^2}{Area} \qquad .. (5)$$

(v) Eccentricity

This parameter is used to measure how much the shape of a nucleus deviates from being circular. The measure is given by Equation (6):

Eccentricity =
$$\sqrt{\frac{a^2 + b^2}{a}}$$
 ... (6)

(vi) Elongation

It is defined as the ratio between maximum distance R_{max} and minimum distance R_{min} from the center of gravity to the nucleus boundary. It is given by Equation (7):

$$Elongation = \frac{R_{max}}{R_{min}} \qquad ...(7)$$

The GLCM features are as follows:

(i) Energy

It is also known as uniformity (or angular second moment). It is a measure of homogeneity in the image.

(ii) Contrast

The contrast feature is the difference moment of the regional co-occurrence matrix and it is a measure of the contrast or the amount of local variations present in an image.

(iii) Entropy

This parameter measures the disorderliness in an image. When the image is not texturally uniform, entropy is very large.

(iv) Correlation

The correlation feature is a measure of regional-pattern linear dependence in the image. The features that best separates the different types of WBCs are used in the fuzzy classification process to determine the count and type of WBC.

3.2.4 Fuzzy Classification

To improve the performance in blood smear classification a new algorithm using fuzzy logic is used. The extracted feature values are used as the input to the fuzzy logic block. The output of fuzzy logic block is a weighting factor to classify the blood smears according to their category. Each linguistic value is represented by an appropriate membership function. The fuzzy rule base is an if...else linguistic rule using the fuzzy input and output sets. This rule base is generated based on expert's heuristic knowledge.

3.3 Classification of Normal/Abnormal WBCs in Blood Smear

For the classification of whether the WBC in blood smear is normal or abnormal, Image enhancement, segmentation and WBC counting are carried out as discussed in Section 3.2 followed by feature extraction. The shape feature that best classifies normal and abnormal blood smears are used in the fuzzy classification process to make the final decision.

3.4 AML Stage Classification

Image Enhancement and segmentation are carried out as discussed in Section 3.2 followed by feature extraction. Some of the tests images used for AML stage classification are shown in Figure 4.

3.4.1 Feature Extraction

Bounding box technique is used to automatically crop the objects in an image. GLCM features discussed in Section 3.2 are used to aid in the fuzzy classification process.



Figure 4. (a) and (b): Test Images used for AML stage classification

4. Experimental Analysis

The dataset used in this paper consist of actual microscopic images of blood samples borrowed from Tirunelveli Medical College Hospital. The images are captured with an optical laboratory Olympus microscope coupled with a Canon Power Shot G5

camera. All of the images are in JPG format with 24-bit color depth and a resolution of 720×570 pixels. The images are taken at a magnification factor of 100x. Also publicly available blood smear images from ASH Image Bank [18] are used. A total of 100 comprising of 50 real and 50 publicly available images are used. The implementation work is carried out using Matlab. So as to evaluate the performance of the proposed work, the metrics namely sensitivity, precision and accuracy are used.

Sensitivity

Sensitivity is also called as recall [15]. Recall is the ratio of the number of samples of a particular sub type classified correctly to the total number of such sub types present in the dataset. Sensitivity is given by Equation (8):

$$Sensitivity = \frac{TP}{TP + FN} \qquad .. (8)$$

Precision

Precision is also called as positive predictive value [15]. It is the ratio of the number of samples of a particular sub type classified correctly to the total number of samples present in the dataset. Precision is given by Equation. (9):

$$Precision = \frac{TP}{TP + FP} \qquad .. (9)$$



Figure 5. (a) - (e): Some test images used for Platelet Count Determination

Accuracy [15] is the percentage of test samples classified correctly by the classifier. Accuracy is calculated as in Equation (10):

$$Accuracy = \frac{TP + TN}{TP + TN + FN + FP} \qquad ...(10)$$

where *TP* is True Positive which is the correct samples correctly classified, *FP* is False Positive which is the correct samples identified as incorrect, *TN* is True Negative which is the incorrect samples identified as correct and *FN* is False Negative which is the incorrect samples identified as incorrect.

In the experiments, platelet count estimation is done as discussed in Section 3.1. Some of the test images used are shown in Figure 5 and the results are shown in Table 1.

Image	Estimated Platelet Count	Classification Result
Image 1	2445000	Thrombocytosis
Image 2	1140000	Thrombocytosis
Image 3	240000	Normal
Image 4	120000	Thrombocytopenia
Image 5	210000	Normal

Table 1. Results for Platelet Count Determination and Classification

WBC Type	Image	Mean	Standard Deviation	Peri meter	Elong ation	Eccen tricity	Solidity	Area
	Image 1	58.65	86.52	300.5	55.05	0.73	0.6	2381
Neutrophil	Image 2	47.63	50.39	168.95	47.52	0.32	0.8	1774
	Image 3	28.65	61.98	157.29	38.77	0.88	0.8	1181
	Image 1	50.28	61.95	185.92	55.35	0.58	0.9	2407
Lymphocyte	Image 2	48.81	56.18	174.85	52.06	0.49	0.9	2129
J	Image 3	52.42	67.55	202.26	59.2	0.63	0.9	2753
	Image 1	36.64	65.28	172.71	47.57	0.82	0.9	1778
Monocyte	Image 2	38.55	71.23	196.95	44.25	0.84	0.7	1538
	Image 3	42.62	60.76	180.46	48.58	0.71	0.8	1854

Table 2	. Values	for	Shape	features	of Sample	WBC types
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WBC type classification is done as discussed in Section 3.2. The values of Shape features and GLCM features recorded for some of the WBC test images like Neutrophil, Lymphocyte and Monocyte are shown in Table 2 and Table 3 respectively.

WBC Type	Image	Energy	Contrast	Entropy	Correlation
Neutrophil	Image 1	0.36	0.29	1.38	0.92
	Image 2	0.23	0.99	1.87	0.89
	Image 3	0.22	0.9	2.02	0.85
Lymphocyte	Image 1	0.49	0.44	1.07	0.87
	Image 2	0.42	0.68	1.37	0.89
	Image 3	0.41	0.57	2.06	0.93
Monocyte	Image 1	0.26	0.81	1.79	0.85
	Image 2	0.29	0.64	1.77	0.91
	Image 3	0.25	0.56	1.72	0.92

Table 3. Values for GLCM features of Sample WBC types

Image	WBC Count	WBC Type Classification		
innige	Count	Neutrophil	Monocyte	Lymphocyte
Image 1	3	1	0	2
Image 2	8	6	2	0
Image 3	7	2	4	1
Image 4	9	4	3	2
Image 5	7	3	2	2

Table 4. Results for WBC type Classification

From Table 2 and Table 3 it can be observed that not all the features contribute much towards the classification process. Hence, only features like as area, solidity and energy are extracted to classify the WBC types. Fuzzy rules are framed such that to classify the type as Neutrophil, the threshold for solidity is fixed as 0.8, and an energy value of greater than 0.40 to classify lymphocyte and a value less than 0.30 to classify Monocyte. The results for WBC cell type classification for some of the test images are shown in Table 4.

Classification of whether the WBC in given bloods smear is normal or abnormal is carried out as discussed in Section 3.3 and the values obtained for various shape features for some test images are recorded in Table 5.

From Table 5, it is observed that the shape feature contributes much towards the normal/abnormal classification process. Of the features extracted, area is used in the fuzzy procedure to make the classification as normal/abnormal.

AML stage classification is done as discussed in Section 3.4 and the GLCM fetaure values for sample test images are repo	orted
in Table 6.	

	1	Area Solidity Perimeter		Solidity		Eccentricity		Elongation		
Image	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Image 1	2129	6216	0.9708	0.7908	174.8528	285.8823	0.495	0.8578	52.0646	63.9901
Image 2	2407	6177	0.9694	0.7651	185.9239	477.6224	0.5841	0.811	55.3596	88.6837
Image 3	2381	6235	0.6601	1	300.5097	54.3848	0.7352	0.5561	55.0598	17.2977
Image 4	1255	6136	0.9554	0.6326	156.8701	480.0488	0.8841	0.8741	40.4459	88.3889
Image 5	1063	2664	0.8247	0.9231	160.9533	211.196	0.9458	0.6548	36.7893	58.2401

Table 5. Values of Shape features for WBC normal/abnormal classification process

Image	Con	trast	Ene	rgy	Entropy		Correlation	
	M1	M3	M1	M3	M1	M3	M1	M3
Image 1	0.5439	1.9983	0.2677	0.1343	1.7994	2.6083	0.8588	0.8859
Image 2	0.5708	2.2305	0.3095	0.3338	1.8319	1.6108	0.9294	0.9251
Image 3	0.22	3.5977	0.5435	0.3402	0.8826	1.82	0.6327	0.8685

Table 6. Values of GLCM features for AML Stage Classification

From the Table 6 it is observed that the GLCM feature contributes much towards the AML stage classification process. Of the features extracted, contrast feature is used in the fuzzy procedure to make the classification as stage M1 or M3.

Metric	Platelet Count Estimation	WBC Count and type Classification	Normal/Abnormal Classification	AML Stage Classification
Sensitivity	1	1	1	1
Precision	0.1	0.94	0.1	0.94
Accuracy (in %)	100	94	100	94

Table 7.	Performance	of the	Proposed	Method
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The Sensitivity, Precision and Accuracy values of the proposed work for Platelet count estimation, WBC count and type classification, normal/abnormal WBC classification and AML stage classification are recorded in Table 7. Thus the proposed work guarantees 100% accuracy for platelet count estimation and WBC normal/abnormal classification and 94% accuracy for WBC type classification and AML stage classification. The results shown in this section have been validated by doctors. Hence the proposed work could be a cost effective alternative for the analysis of Blood smears.

5. Performance Comparison and Discussion

The proposed method of platelet counting from blood smears is fast, cost effective and accurate to produce blood cell report. It gives 100% accurate results which is an important criterion to diagnose diseases like dengue at an initial stage. The time and cost limitations associated by conventional techniques are overcome by this method, as well as an estimate of the range in which the platelet count may lie is accurately predicted. None of the work has been reported so far for platelet counting with the available literature. Hence, this technique is extremely suitable in the present scenario where 100's of samples are collected each day and hence, the time and cost factors are crucial while predicting platelet estimates.

Anjali Gautam et al. [13] have developed a method for the classification of WBC as Neutrophil, Eosinophil, Basophil, Monocyte and Lymphocyte and the accuracy of their method is 70%, 62.5%, 70%, 50% and 100% respectively as shown in Table 8 and processing time is compared in Table 9. Different types of structuring elements are used to have an effect on the shape of WBC's. However, the proposed system could make classification of WBC types at an accuracy of 100% for Lymphocyte, Neutrophil and Monocyte and at an accuracy of 50% for Eosinophil and Basophil with an average processing time of 15 seconds.WBC cell type classification will help the clinician in further diagnosis to count all types of components in the blood at the same time.

	Classification A	Accuracy (in %)
WBC Type	Existing System [13]	Proposed System
Neutrophil	70	100
Eosinophil	62.5	50
Basophil	70	50
Monocyte	50	100
Lymphocyte	100	100
		1

Table 8. Comparison of Existing and Proposed System on WBC cell type Classification

Method	Average Processing Time (in Seconds)
Existing System [13]	0.067374
Proposed System	15

Table 9. Comparison of Existing and Proposed System on Average Processing Time

Sos Agaian et al. [19] have developed a method using features like Hausdroff Dimension (HD), Local Binary Pattern (LBP), Gray Level Co-occurrence Matrix (GLCM) and shape features to make a decision as to classify the input blood smear as normal and abnormal. The accuracy of their system is reported to be 93%. However, the proposed system proves to be efficient with an accuracy of 100% with a minimal set of features to classify the WBCs as normal or abnormal and it is shown in Table 10.

As a standard procedure, the hematologists diagnose Acute Myeloid Leukemia (AML) stage based on cytogenetic testing. Earlier work using image processing involves testing the subtypes through genetic algorithm a way of testing the chromosome

in the blood cell [20]. The system has given less accuracy due to its classifier performance. Proposed feature selection and fuzzy classification procedure could make AML stage classification with an accuracy of 94% as against the existing system with an accuracy of 70.59%. This is given in Table 11.

Method	Classification Accuracy (in %)
Existing System [19]	93
Proposed System	100

Table 10. Comparison of Existing and Proposed System on Classification of Normal and Abnormal Blood Smears

Method	Classifier Used	Accuracy (in %)
Existing System [20]	Naive Bayes	70.59
Proposed System	Fuzzy Logical Approach	94

Table 11. Comparison of Existing and Proposed System on AML Stage Classification

6. Conclusion

In this work, platelet in blood smears is extracted and estimated by edge detection, morphology based segmentation and calculation. Also, the type of WBC cell, whether the WBC in blood smear is normal or abnormal and what stage of AML is if abnormal are classified using a fuzzy classifier with enhancement and segmentation prior to that. Suitable features which aid in the classification process are analyzed and reported. This work will eliminate the manual efforts by doctors and aid in automated analysis of blood smears. Particularly it saves time and minimizes cost. The methods for platelet count estimation and normal/ abnormal WBC classification guarantees 100% accuracy and platelet count estimation could be used as a preliminary screening for dengue which is predominant in this geographical region. The approaches used for WBC cell type and AML stage classification guarantee an accuracy of 94 %. Future work is on predicting other blood related disorders from blood smears.

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