ISSN: 0974-5823 Vol. 6 No. 3 December, 2021

International Journal of Mechanical Engineering

Estimation of Platelet Count from Peripheral Blood Smear Slide Images

B. Padmapriya

Associate Professor, Department of Biomedical Engineering, PSG College of Technology, Coimbatore, Tamil Nadu, India

***Abstract*— The aim of this study is to evaluate the performance of image processing algorithms for automatic, rapid screening and counting of platelets from peripheral blood smear slide images. Peripheral smears prepared from blood samples of a group of 100 patients of varied age group including children of age below 15, underwent the process of automatic estimation of platelet count. The patients were grouped into three categories based on the platelet count, namely normal, low risk and high risk. The sensitivity, specificity, accuracy and correlation coefficient were calculated for the classification of each patient into the appropriate category. The automated methods for platelet counting are found to be clinically useful for rapid screening of smear slide images.**

Index Terms*— *platelet count estimation, peripheral blood smear slide, color segmentation, K-means clustering, rapid screening.

1. INTRODUCTION

Platelets are very small, non-nucleated cellular components of blood, about 3 μm in diameter, and consisting of cytoplasm enclosed within a cell membrane [1]. In Leishman stained smears, platelets appear circular with a dark purple color as seen in Fig 1.

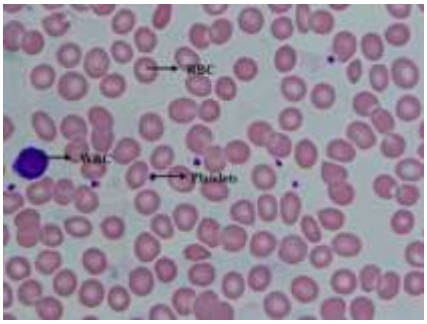

Fig.1. Platelets in Leishman Stained Smear

The normal range of platelet count in a healthy individual is 1, 50,000 – 4, 00,000/μL [2]. These numbers can drop significantly, leading to thrombocytopenia (<1, 50,000/μL) if a patient is suffering from underlying conditions. The causes for thrombocytopenia can be broadly classified into three types viz, hypo productive thrombocytopenia, hyper destructive thrombocytopenia and thrombocytopenia secondary to sequestration. The incidence of fevers such as Dengue, Malaria, Yellow fever, and others are associated with thrombocytopenia which is usually of hyper destructive etiology [3].

Platelet count estimation is one of the vital tests performed in healthcare centers using automated cell counters or by a manual blood smears examination through a microscope. Whilst the former is not cost effective, the latter method is laborious and is associated with intra and interobserver variations. In order to curtail costs and to provide reliable platelet counts, image processing techniques can be used to develop rapid screening and counting of platelets from blood smear images [6].

Image processing algorithms have been proposed in literature for automatic, rapid screening and counting of platelets from peripheral blood smear slide images. Simple algorithms which are based on gray scale thresholding are computationally effective for rapid testing. However, the image quality of blood smear images are not consistent due to several factors such as variation in microscope's light source,

variation in the exposure and white balance of imaging sensors which lead to differences in colour, contrast and brightness of images. Hence it is not possible to optimize a single threshold value for different blood smear images. Segmentation techniques using morphological operations and blob processing techniques are proposed; however these methods do not give optimal results when platelet overlaps with RBCs [7] or artifacts such as foreign particles and excessive staining. Fuzzy algorithms provide good results in comparison with the other simple and complex algorithms. However the algorithms are complicated and not suitable for rapid testing. Colour based algorithms and k-means clustering based algorithms offer good segmentation accuracy and also ease of implementation.

For the purpose of this study, color based segmentation algorithms were evaluated for their efficiency in accurate segmentation of platelets, followed by an automated count of the number of platelets. The patient group taken up for the study constituted 100 subjects of varied age including children of age below 15. The ethical clearance formalities were duly completed and the patient data was provided for a

double – blind study by the Clinical Pathology & Haematology Division of the Pathology Department of PSG Hospitals, Coimbatore.

The results of platelet count obtained from Coulter cell counters were documented for comparison with the automated algorithm. Based on the platelet count, three groups of study population were arrived at viz., 1) Platelet count in normal reference range (ie > 1,50,000/cu.mm) 2) Platelet counts between 50,001 and 1,49,999 /cu.mm & 3) Platelet counts equal to or below 50000/cu.mm. Peripheral blood smears were made from all the blood samples and stained by Leishman stain. Image acquisition and image analysis using two algorithms were performed to obtain the platelet count.

2. Platelet Count Estimation Using Conventional Methods

For a blood smear examination, 10 oil immersion fields were counted and the results were averaged and the count estimated using equation (1)

$$\text{Estimated platelet count/}\mu\text{L} = \text{average count in 10 fields} \times 15{,}000 \quad (1)$$

For counting of platelets using a haemocytometer, 50 µL K2EDTA blood is mixed in 950 µl dilution platelet counting fluid solution to give a dilution of 1:20. This solution has a lysate, which lyses the RBCs and a dye such as Brilliant Cresyl Blue, which stains the platelets, and WBCs. Once the erythrocytes are completely lysed, the suspension is mixed and put into the counting chamber. Calculation of the platelet count is achieved by using equation (2)

$$\text{Platelets} / \mu\text{l} = \frac{\text{Number of counted platelets}}{\text{Number of counted platelets} \times \text{Dilution of the cell solution}} \quad (2)$$

Automated haematology analysers count platelets by electrical impedance or optical density or both. In both these principles, the blood sample is mixed with a lysate which lyses the RBCs keeping the WBCs and platelets intact. These cells are then passed through an aperture as a single cell flow. The aperture has either an electrical current or light beam passing through it and whenever any cell passes through, it creates impedance which is converted into electrical signals and represented as a histogram. The height of the pulses is analyzed to estimate the platelet count while other parameters such as mean platelet volume and plateletcrit are derived from the histogram.[9].

3. PLATELET COUNT ESTIMATION USING IMAGE SEGMENTATION TECHNIQUES

Blood samples were collected from the volunteers after ethical clearance obtained from PSG Hospitals and consent from the patients. Peripheral smears were created and images captured using optical laboratory Olympus BX-53 microscope coupled with a Q-imaging 3.3 MP camera. The images were taken at a magnification factor of 100x (Oil immersion). Conventionally platelets are counted from 10 fields in clinical practice. The average number of platelets in all the 10 fields is estimated and the average value is multiplied by a factor of 12,000. However in case of processing the images using automated algorithms, 20 fields of view were suggested by the pathologists. Further the Field of View (FoV) captured by the camera used in this work was only 30% of the entire field. Hence 3 images needed to be captured for being equivalent to one field. This will require 60 images to be captured to be equivalent to 20 fields of view. From each smear slide 20 microscopic images were captured and the image data was stored using the code assigned to each sample. The images were captured from 100 slides accounting to 2000 images. The real time microscopic images consist of several noise and artifacts. A median filter is used to enhance the image [10]. A window size of 11 helped in removing the excess stain particles, but at the same time preserving useful information.

3.1. Colour based segmentation technique

The microscopic images were captured in the RGB format and conversion was done to HSV colour space and the three colour planes are shown in fig 2. The platelets are seen brighter against darker background in S plane while the platelets are not evidently seen in other planes.

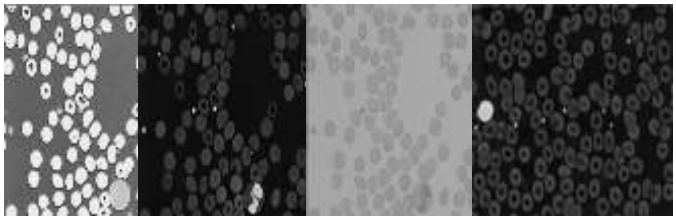

Figure.2. (a) H plane (b) S plane (c) V plane (d) S plane

Hence the S plane is used for further processing. The extraction of platelets from the S plane is done through two consecutive thresholding operations. The first thresholding operation is based on the intensity of the components in the image [11].

$$g(x,y) = \begin{cases} 1 \text{ if } f(x,y) > T \\ 0 \text{ if } f(x,y) \le T \end{cases} \quad (3)$$

With a threshold of t= 115, using equation (3), the platelets and WBCs that are stained in the same colour and hence take up similar intensity range are segmented from the rest of the image as seen in fig 3(a). It can be noticed that in the resulting binary image, the only parameter that differentiates the platelet from WBC is the size. Platelets are seen to exist in the size of 150 – 30,000 pixel^2. Circularity parameter with a circularity range of 0.5 – 1.0 was also used to distinguish platelets from other artifacts. The resulting image is seen in fig 3(b).

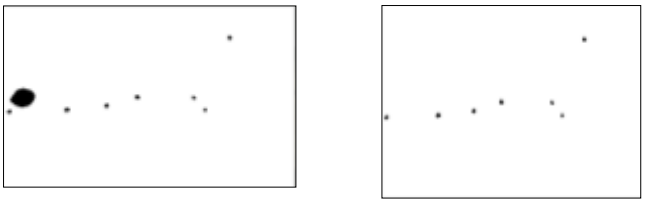

Fig.3. (a)Intensity Thresholding (b) Area Thresholding

3.2. K-Means clustering based segmentation technique

K-Means clustering algorithm forms K clusters such that the distances between the points in a cluster are relatively small compared to the distances to data outside the cluster [12]. To find an assignment of the data to clusters that minimizes the sum of squared distances of each data point to its closer prototype or cluster mean μ_k. The process stops when no movement of data from a cluster to another reduces the objective function J or a maximum number of iterations is reached. The objective function is the sum of squared distances for each data point x_n to its corresponding cluster prototype μ_k and it is given by equation (4).

$$J = \sum_{n=1}^{N} \sum_{k=1}^{K} r_{nk} \| X_n - \mu_k \|^2 \quad (4)$$

The algorithm clustered the components in each image into two groups. One cluster contained the WBCs and platelets while the other cluster contained RBCs and the background as shown in Fig 4(a) and (b).

The cluster with platelets and WBCs was taken up for further processing. Area thresholding was done to segment the platelets [13].

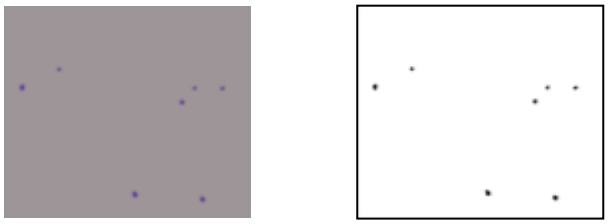

Fig.4.(a) K-Means clustering algorithm (b) Image with segmented platelets

3.3. Platelet count estimation

The platelets in the image can be now counted. For each slide, 20 images were processed using batch and equation (5) was used to determine the platelet count of the patient [14].

$$\text{Estimated Platelets count / µl} = \frac{\text{Number of platelets in 20 fields}}{20} \times 12{,}000 \quad (5)$$

Using the total sum of platelets in 20 fields, the average number of platelets per field is estimated which is then multiplied by a factor 12,000 to get the platelet count of the patient [15].

4. STATISTICAL ANALYSIS

The images of the peripheral blood smear were analyzed using the two segmentation techniques to obtain the platelet counts. Results of platelet counts obtained by these two algorithms were compared with those obtained using the automated blood cell counter and analyzed using paired T-test. The results of mean platelet count as per the category is given for the entire 100 samples in table 1

TABLE 1. MEAN PLATELET COUNT AS PER THE CATEGORY

Platelet count group	No. of cases	Mean Platelet count		
		AHA	*Algorithm 1*	*Algorithm 2*
> 1,50,000	58	3,27,137	3,24,625	3,38,263
50,001-1,49,999	9	1,05,555	1,10,600	1,21,044
≤ 50,000	33	27,818	28,042	31,072

As patients with platelet count < 50,000 are more prone for bleeding than those with counts > 50,000, comparative results of this group are displayed below in table 2.

TABLE 2. COMPARISON RESULTS OF PLATELET COUNTS FROM AHA AND SUGGESTED SEGMENTATION TECHNIQUES

S. No	Platelet count		
	Automated Hematology Analyzer (AHA)	*Color based segmentation technique*	*K-means clustering technique*
1.	6,000	5,600	3,600
2.	9,000	10,200	11,200
3.	10,000	9,000	11,200
4.	13,000	14,400	15,000
5.	14,000	15,000	18,200
6.	16,000	15,000	15,800
7.	16,000	15,000	16,800
8.	16,000	18,400	20,400
9.	20,000	22,800	24,800
10.	21,000	20,400	22,400
11.	22,000	21,400	26,400
12.	22,000	25,800	26,800
13.	23,000	24,400	29,400
14.	23,000	24,600	26,600
15.	24,000	24,600	25,600
16.	24,000	25,800	28,800
17.	25,000	22,200	28,400

18.	26,000	26,400	26,000
19.	27,000	25,600	29,600
20.	28,000	30,600	31,400
21.	31,000	28,200	34,200
22.	35,000	32,600	38,400
23.	36,000	35,200	38,800
24.	38,000	40,200	52,000
25.	40,000	41,200	46,200
26.	41,000	41,400	43,400
27.	42,000	43,800	44,400
28.	43,000	42,800	45,600
29.	44,000	43,200	48,200
30.	44,000	40,800	42,400
31.	44,000	43,800	51,600
32.	45,000	44,600	50,600
33.	50,000	50,400	51,200

Results of T-test performed between AHA and Color based segmentation technique is 6.134558. The value of p is < 0.00001. The result is significant at $p \leq 0.05$.

Results of T-test performed between AHA and K-means clustering Algorithm is 6.395183. The value of p is < 0.00001. The result is significant at $p \leq 0.05$.

A statistical analysis is done on the reported count against the estimated counts obtained using the two algorithms implemented. Parameters including sensitivity, specificity, accuracy and correlation coefficients are calculated and tabulated in table 3.

TABLE 3. STATISTICAL ANALYSIS PARAMETERS OF SEGMENTATION TECHNIQUES

Parameter	Color based segmentation technique	K-means clustering based technique
Sensitivity	0.97	0.88
Specificity	1	1
Accuracy	0.99	0.96
Correlation Coefficient	0.994088	0.983818

Based on the analysis of the statistical results, it is evident that the color based segmentation technique (method 1) is more efficient in segmenting and counting platelets than the K-means clustering based technique (method 2) [16].

5. CONCLUSION

The algorithms developed have the advantage of eliminating illumination artifacts and other artifacts like muck and stain. The screening results aid in grouping the patients into the appropriate category of classification. Thus the performance of the algorithms for rapid screening and counting of platelets from peripheral smear slide images is tested and analyzed using various metrics like t- test value, sensitivity, specificity, Accuracy and correlation coefficient. Based on the analysis, color based segmentation technique works effectively and efficiently in rapid screening process.

REFERENCES

[1] *KakaliGhoshal and Maitree Bhattacharyya., "Overview of Platelet Physiology: Its Hemostatic and Nonhemostatic Role in Disease Pathogenesi"s, The Scientific World Journal, 24, 343-359, 2014*

[2] *Malok M, Titchener EH, Bridgers C, Lee BY, Bamberg R., "Comparison of two platelet count estimation methodologies for peripheral blood smears", Clin Lab Sci, 20, 154-160, 2007.*

[3] *Behnke O and Forer A.,"Blood platelet heterogeneity: Evidence for two classes of platelets in man and rat", Br J Haematol, 84, 686-69, 1993.*

[4] *Nurden AT, Nurden P, Sanchez M, Andia I and Anitua E.," Platelets and wound healing", Front Biosci, 13, 3532-3548, 2008.*

[5] *Prabakaran K, Imran Khan F, Mohammed Abrar N, Mohammed Abbas F, Salman Khurshid S., "A Smart Sensing and quantification of Platelets, RBCs and WBCs using Microscopic Blood Image", International Journal on Applications in Medical Science and Research , 1(1), 1-9, 2015.*

[6] *RatnadeepDey, Kaushiki Roy, DebotoshBhattacharjee, MitaNasipuri and PramitGhosh.," An Automated system for Segmenting platelets from Microscopic images of Blood Cells", International Journal of Engineering Trends and Technology (IJETT), 4(7), 2896-2902, 2013.*

[7] *KashmalaIlyas and MehreenSirshar., "Dengue Detection Using Morphological Segmentation techniques and Blob Processing for Automatic CBC", International Journal of Computer and Communication System Engineering (IJCCSE), 2(2), 346-35,2015..*

[8] *Anil K Jain, "Fundamentals of Digital Image Processing", Prentice Hall of India Pvt Ltd., New Delhi, 2009*

[9] *Mohamed-Rachid Boulassel, Raya Al-Farsi, Sulaiman Al-Hashmi, Hamad Al-Riyami, Hammad Khan, and Salam Al-Kindi.,"Accuracy of Platelet Counting by Optical and Impedance Methods in Patients with Thrombocytopaenia and Microcytosis", Sultan Qaboos Univ Med J. 15(4): e463–e468, 2015.*

[10] Brecher G, Schneiderman M, Cronkite EP.,"The reproducibility and constancy of the platelet count", Am J ClinPathol, 23, 15-26, 1953.

[11] Anjali Gautam and Bhadauria HS., "Blood cells Extraction using K-Mean clustering and mathematical morphing", IJEEE, 1(3), 9-12, 2014.

[12] Jayaraman S, Veerakumar T, Esakkirajan S, "Digital Image Processing", Tata McGraw Hill, New Delhi, India., 2009.

[13] Sai Prasad A, Surya Latha K, KoteswaraRao S., "Separation and Counting of Blood cells using Geometrical Features and Distance Transformed Watershed", International Journal of Engineering and Innovative Technology (IJEIT), 3(2), 77-82, 2013.

[14] Bakhubaira S., "Automated versus manual platelet count", J ClinExpPathol, 3, 3-6, 2013.

[15] BartoszKrawczyk., "Automatic detection and counting of platelets in microscopic image", Journal of Medical Informatics & Technologies, 16, 173-178, 2010.

[16] Weixing Wang, Hao Song and Qi Zhao., "A modified Watersheds Image Segmentation Algorithm for Blood Cel"l, International Conference on Communications, Circuits and Systems, 1, 450-454, 2006.