Case Report

AUTOMATED DIFFERENTIAL BLOOD COUNT USING IMAGE QUANTIZATION

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ABSTRACT

Objective: The objective of this research is to automate the calculation of differential blood count in blood smear photomicrographs using image quantization.

Material and Methods: A series of image processing steps were used for the detection of White Blood Cells (WBCs), Red Blood Cells (RBCs) and platelets as: image acquisition, separating the channels of RGB and applying wiener filter on each channel for smoothing the image. The purpose is to enhance the visual interpretation of the image, recombining the channels and applying the quantization over the wiener output.

Results: The accuracy of this technique is very close to that of the hematologists' manual calculation. It was 85% for Red blood cells and 98% for White blood cells.

Conclusion: This proposed technique gives precise results under varied luminance conditions such as darkness, brightness and low contrast images, it gives reliable results for all the images in the image sets having different quality of images.

Key Words: Blood Smear, White Blood Cells, Red Blood Cells, Platelets, hematology, Quantization, digital image processing.

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INTRODUCTION

Many diseases can be diagnosed and evaluated by counting and classifying the blood cells in the microscopic blood smear images. The prominent disorders which are frequently diagnosed in the blood samples of most patients are leukocytosisand leucopenia. After categorizing a blood smear as White Blood Cells (WBCs) with lower or higher counts, they are further classified into various diseases according to hematology's own protocols[1]. Similarly, the diseases related to the erythrocytes can also be found in the human blood samples studying some changes like increase or decrease in the size or count of RBC's. Malarial parasites in the blood affect the compactness of cells which varies from that of normal cells, or if there is low RBC count then the disease is labeled asanemia.

This research work has an inspiration from the previous work done by Bakht A.[2] In our previous work, the WBCs were detected and counted automatically by quantizing the digital blood smear photomicrographs. The protocols used by the hematologists were used as part of algorithm of our work. WBCs were collected as filtered residue and the rest of the contents were vanished. The labeled WBC's were then counted up and were matched with the results produced by the hematologists manually.

As revealed previously, hematologists used to count the main components in the blood smear slides. A normal man and woman has its normal range, shown in Table 1, and mentioned in the protocols build by the World Health Organization WHO.[3] If a count exceeds or lowers than the normal range, then it will be considered as abnormal and a further action will be taken by the physician after interpreting it. In Figure 1, different types of cells are visible in the image, the large cell having nucleus in it, is WBC, the disc shaped cells, having no nucleus smaller than WBC is RBC and the smallest objects are the Platelets.

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These distinguishing features of the above cells are used for the segmentation purpose of the blood smear images and keeping in view the following counts given in Table 1, decisions are taken whether the count is normal or not.

MATERIAL & METHODS

This research work is categorized into two stages. In the first stage, the input image is preprocessed and the WBCs are segmented. This segmented image become an input for the second stage. In the second stage, the WBC segmented image is processed and gives output as RBC's,

Stage 1 (WBC Segmentation)

Image acquisition:

A special microscope with a high resolution camera mounted over it, used for acquiring the blood smear photomicrographs. Snapshots were taken of different fields, an oil immersion 100 x objective was used, and the resolution of images was kept 750 x550 pixels. Figure 2 shows the step by step approach of this algorithm and is explained as follows:

Preprocessing

In the next step the acquired images were preprocessed[4]. The images having uneven factors like increased luminosity, darkness and brightness were normalized and a wiener filter of window size 7 x 7 using the following equations was applied over the images in the manner shown in Figure 3.

$$= \frac{1}{NM_{n1,2\in\eta}} a(n_1, n_2) \quad (1)$$

$$\sigma^2 = \frac{1}{NM_{1,2\in\eta}} an12 - \mu^2 \quad (2)$$

Equation (1) and (2) calculates the mean and variance around each pixel respectively.

Where η is the N-by-M local neighborhood of the candidate pixel of an input image in the following manner. This filter generates a range of frequencies from an image and automatically cuts off the filter at frequencies where noise becomes significantly higher than signal.

Quantization

Quantization actually means to reduce the number of levels[5]. Here we are reducing the number of color levels to such an extent that desired components (WBC's) remain visible while the other contents lose their visibility as shown in Figure 3, this is the backbone of our research work, and can be more clarified from Figure 4. First, the input image is discriminated into R, G and B channels then a wiener filter is applied individually over each channel producing wienered IR, IG and IB. A factor F was set to a value of 255 after an iterative process, observing good results of visibility of WBC's and disappearance of the other contents, as shown in Figure 5.

Binarization

We used Otsu thresholding method[6] (use consistent voice either passive or active) for binarizing the quantized image, which dynamically calculates a threshold value from the input image and classify the pixels as follow:

$$o^2\omega(t) = \omega(t_1)\sigma^2(t_1) + w(t)_2\,\sigma^2{}_2(t) \quad (3)$$

$$\omega_1(t) = \sum_{i=1}^{t} p(i) \quad (4)$$

$$\omega_2(t) = \sum_{i=t+1}^{t} p(i) \quad (5)$$

$$\mu_1 t = \frac{t}{t=1}\frac{iPi}{\omega_1 t} \quad (6)$$

$$\mu_2 \frac{1}{1=t+1} = \frac{iPi}{\omega 2t} \quad (7)$$

Where equation (3) shows the weighted sum within-class variance, equation (4) and (5) shows how the class probabilities are projected, the class means are calculated as in equation (6) and (7) and finally the individual class variances are calculated as in equation (8) and (9):

$$O^2{}_1(t) = \frac{t}{t+1} i - \mu_1(t)^2 \frac{P_\omega(i)}{1(t)} \quad (8)$$

$$O^2{}_2(t) = \frac{t}{i=t+2} i - \mu_2(t)^2 \frac{P_\omega(i)}{2(t)} \quad (9)$$

$O^2{}_1$ (t) = The variance of the pixels in the background (below threshold)

$O^2{}_2$ (t) = The variance of the pixels in the foreground (above threshold), Weights ωiare the probabilities of the two classes separated by a threshold t and $\sigma^2{}_i$ is the variances of these classes.

MorphologicalOperations

In order to make the extracted objects in the image closest to the shapes of the natural objects, these operations are carried out, the morphological operations we have done are erosion, dilation, opening and then reconstruction.

Opening is the dilation of the eroded set A by a structuring element B:

$$A \circ B = (A \varnothing B) \oplus B \quad (10)$$

Where o standsforopening, ∅representserosionand ⊕ denotesdilation.

This operation was carried out, in order to make the edges of the extracted components smoother and clear the noisy artifacts[7].

WBC'sSegmentation:

WBC's segmentation is the final step of stage 1 of this methodology, which were easily distinguished from other objects (RBC's and Platelets) leveraging its color feature in the quantization step of this technique explained in Figure 2.

In Figure 5, (a) is a cropped part of an original image, containing a WBC and some RBCs, (b) is the wienered image, (c) is the quantized image showing a WBC and the eliminated RBC's, (d) is binarized form of (c) and (e) is the labeled WBC.

Stage 2 (RBCSegmentation)

WBCMasking

The WBC's extracted in the final step of stage 1 are used as masks for the input image (binarized form of original image) in stage 2, which was overlayed upon the binarized image of the original image and pointing the said WBC's.

Image Subtraction

After using mask of WBCs, it is easy to subtract it from the binarized image of original one, the subtraction process results in the disappearance of WBCs and remaining out the RBCs as shown in Figure 6.

Mathematical Morphology:

The shape of RBC is biconcave, flattened and disc like structure, having no nuclei in it. Its average size is 6-8 μm in diameter. These features are kept in mind in order to eliminate unwanted artifacts or roughness in the edges of contents left behind after masking and image subtraction process. Mathematical morphological operators like closing and opening are used.

Area Filtering:

RBCs are of average size in all the contents present in human blood, this size of RBCs helped in recognizing them and the extra artifacts were filtered out in this step.

$$\text{Area Filter} = AF(\sigma 2 - w(t), p) \qquad (11)$$

RBC's Segmentation:

Finally, the residual components were RBCs, after all operations being performed on blood smear images and which were successfully segmented from the rest of image. The result is show in Figure 6.

RESULTS

The proposed technique was tested on 4 image sets, each containing 15 images, prepared by authors in the department of pathology of a local postgraduate research hospital. The image sets were manually examined by well qualified pathologists and each image was commented bythem.

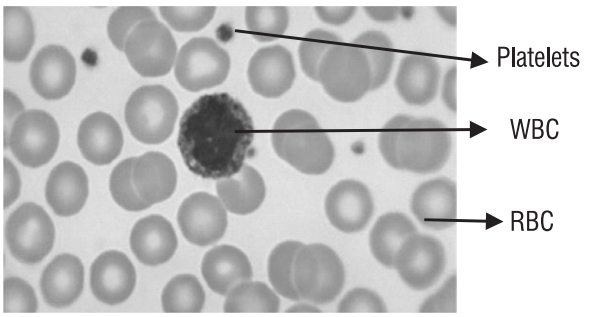

Figure 1: A sample blood smear showing RBC, WBC, Platelets

Table 1: Normal Blood Count of a human adult[1]

Type of cells	Gender (adults)	
	Male	Female
Red Blood Cells (RBC)	4.5-6.0 million / Microlitre	4.0-5.0 million / microlitre
White Blood Cells(WBC)	4.5-11 thousand/ microlitre	4.5-11 thousand/microlitre
Platelets	150-450 thousand/microlitre	150-450 thousand /microlitre

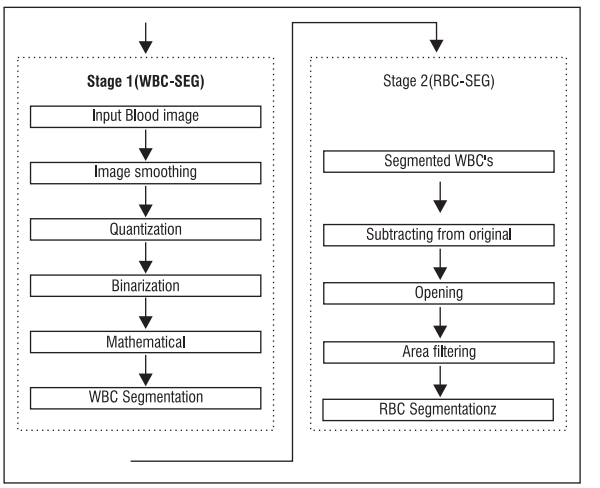

Figure 2: Block diagram of the proposed technique

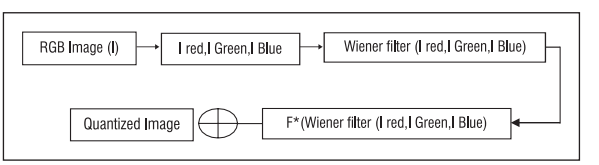

Figure 3: Wiener filter followed by quantization[2]

Each image set was evaluated through our proposed algorithm. The results were cross matched with some other novel techniques and showed better accuracy and reliability in many aspects as compared to them.

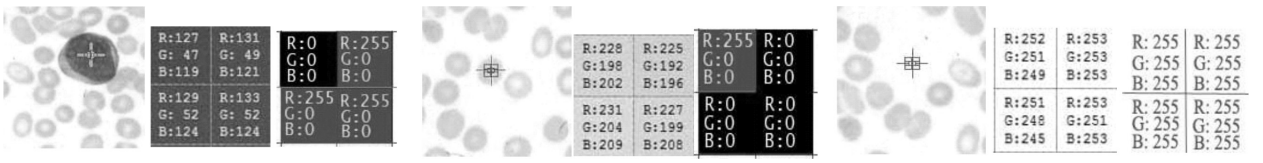

Figure 4: Sample pixel values after quantization (a) WBC, (b) RBC, (c) Background

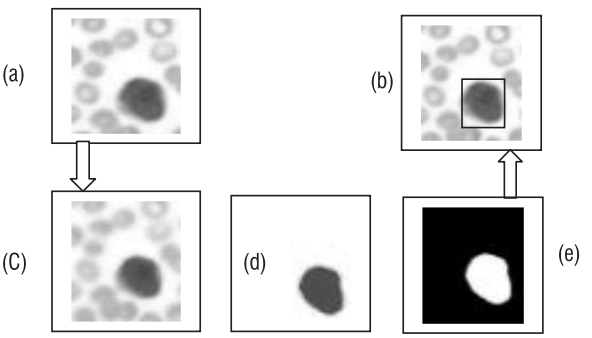

Figure 5: WBC's segmentation in stage 1

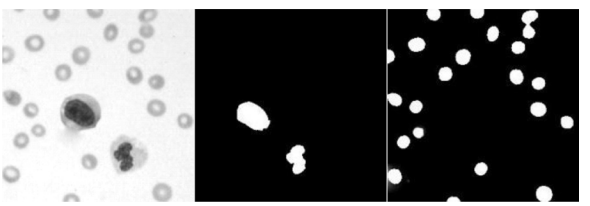

Figure 6: a) Originalimage b) SegmentedWBCs c) SegmentedRBCs

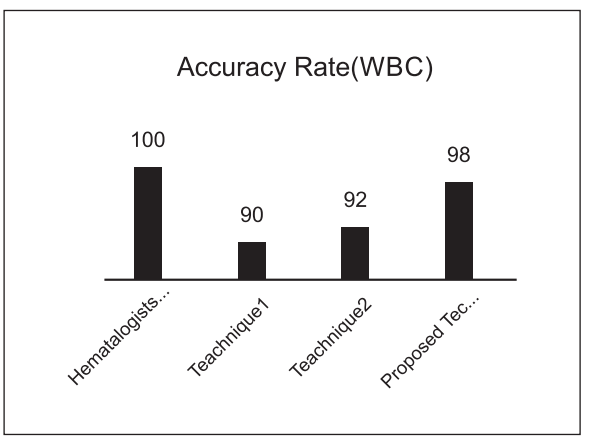

Figure 7: A graph showing the accuracy rate for WBC's of each technique (technique 18 technique 29and the proposed one)

The accuracy rate of this technique was determined by comparing the count done by us with the pre calculated counts of cells done manually by expert hematologists using the following formula:

This table shows the true and false cells detected by each technique. Technique 1 and technique 2 has detected one cell for image 1 in image set 4, technique 2 has detected one cell in image 4 and one cell in image

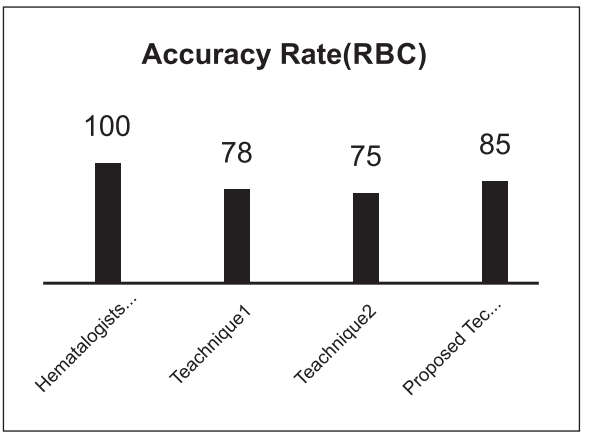

Figure 8: A graph showing the accuracy rate for RBC's of each technique (technique 18, technique 210and the proposed technique)

9, which has greatly suffered the efficiency of these technique while there is no false cell detected by our proposed technique.

DISCUSSION

A number of techniques for processing the blood smear images and counting mechanisms have been developed. Diaz G and Hiremath PS presented a method for the extraction of RBC's in the peripheral blood smear images infected with Plasmodium Falciparum.[11,12] They utilized Gradient Vector Flow (GVF) in order to extract out the white blood cells and then used zack thresholding to separate cytoplasm from the nucleus.[13] Another advanced work is also done to classify WBCs automatically by computing the WBC images in term of area, major axis length over minor axis length, perimeter, circularity and ratio of areas between nucleus and cytoplasm.[14] Joshi MD used contrast stretching and median filter for image enhancement and Otsu thresholding for binarization.[15] Various morphological operation like opening and closing were used for removing the extra artifacts other than WBCs.[15]

Putzu L used histogram equalization followed by contrast stretching for image enhancement and watershed for separating overlapped cells for the identification of WBCs in blood smear images.[16] Mahmood NH for the purpose of segmentation of blood cells used color based segmentation technique followed by the canny edge detection technique and segmented the image

Table 2: WBC/RBC detected in one of the image sets.

Image #	True Cells detected							
	Hematologist		Tech 16		Tech 27		Proposed Technique	
	WBC	RBC	WBC	RBC	WBC	RBC	WBC	RBC
1	3	43	4	30	4	32	3	35
2	4	39	4	28	4	29	4	32
3	6	32	5	25	4	25	6	27
4	6	30	5	20	7	20	5	22
5	8	38	7	30	7	32	7	34
6	7	40	7	28	6	27	7	32
7	6	29	5	20	5	21	6	22
8	9	31	8	21	8	21	9	24
9	4	24	4	17	5	19	4	23
10	10	49	8	32	9	31	10	35
11	11	47	10	33	11	30	11	35
12	5	39	5	29	5	30	5	33
13	9	56	8	41	8	43	9	47
14	1	33	1	23	1	23	1	30
15	13	50	12	37	12	38	12	39

using circular Hough Transform with ROI polygon area measurement.[10] Jambhekar ND calculated the image gradient through the derivative of filter of Gaussian and detected the edges through the local maxima of that gradient image in order to segment RBCs from the rest of image.[17] Nasrul A used a 5x5 median filter on the green component of original image with contrast stretching technique for enhancing the contrast between RBC and WBCs for the easy separation of RBCs and used saturated component of HSV to extract WBCs.[10] Tulsani H used a 3 x 3 average filter for smoothing in YCbCr color space and morphological operations followed by watershed algorithm for the segmentation of RBCs, WBCs and platelets[18]. Hazwani M extracted the saturated component from the HSV color space and applied a 3 x 3 median filter on it[18]. Then the histogram of the S component was plotted in order to determine the threshold values, they selected 110 as the threshold value on the S component of acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) images and separated the infected WBCs from the rest of image, this technique is also helpful in counting purpose. Sharif JM converted the original image into YCbCr color space and choose the second component of YCbCr, as almost all information relating classification of WBC's are present in it, and is used specially for the normalization of illumination that affect the quality of the image.[19] They binarized each component separately and divided the Cb component by Y and extracted WBCs in thisway.

The above techniques discussed have contributed a significant role in the field of segmentation, but the only shortcoming in almost each of them is that of non-consistent behavior with respect to change in image conditions, which is up to much extent eliminated in our proposed technique.

The only problem we faced during the experimental phase of our research was that the accuracy of our technique was highly suffered by overlapped cells. Leveraging our previous work combining the current methodology, we aim to build an automated system that will classify and quantify the complete blood contents of a peripheral blood smear images and will detect many severe diseases like Acute Myeloid Leukemia, Chronic Lymphocytic Leukemia etc. However, large prospective, multicentre trials are required for generalization of these findings.

CONCLUSION

Proposed algorithm is quite simple, efficient and accurate. The authors have tested many images under different luminance conditions like darkened, brightened and low contrast images, it gives consistent results for about entire image sets that were created.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Azam B: Study design, data collection, data analysis.

Rahman S: Literature review.

Alam F: References, Bibliography, statistics, data analysis.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.