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An Automated Method for Counting Red Blood Cells using Image Processing

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Abstract

In healthcare, blood testing is observed to be as one of the most significant medical examination tests. In pathology labs, different types of blood cells are counted to diagnose the diseases patient. By counting RBCs (Red Blood Cells) in images of blood cells can play a very great role in detection as well as to follow the treatment process of number of diseases such as anemia, leukemia etc. Counting and examination of blood cells manually by microscope is tedious, time intense and entails a lot of technical expertise. Hence arises a need to come across for automated blood cell detection and counting system that can facilitate physician for diagnosing diseases in fast and efficient way. According to present studies, the RBCs are classified in four types of abnormality, namely elliptocytes, echinocytes, tear drop cells and macrocytes. In this paper, technique has been introduced to count the RBCs automatically. In proposed work, images are classified on the basis of color, texture and morphology. Process of counting of cells is done into three parts: image processing including texture feature extraction using morphology, thresholding segmentation and counting of cells using Hough transformation. The proposed algorithm achieves overall accuracy of 91.667% and is computationally very efficient as it takes only 0.81432 seconds to count the number of red blood cells for different blood samples.

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1. Introduction

Around the globe, different lives are gradually influenced by innovation, with healthcare being the primary areas for

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these changes. Providing instant details of patient's well being history enhances patient's alert through correct diagnosis and medicines, the ability to share health information to different doctors, and the reduction of mistakes and errors found when recording patient health information data through manual records.

Blood is a connective tissue composed of specialized cells suspended in a liquid medium, the plasma. The cellular components of blood comprise of three kinds of cells i.e. the red blood cells (erythrocytes), the white blood cells (leucocytes) and the platelets (thrombocytes). These groups can be discriminated using color, texture, size and the morphology of nucleus and cytoplasm. The primary function of red blood cells (RBC) is to transport oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs [1] [2]. Each RBC is a non-nucleated, biconcave disc with a mean diameter of approximately $7.5\mu\text{m}$ and thickness of about $2\mu\text{m}$ at the periphery and about $1\mu\text{m}$ in the middle. The kinds of ailments related to RBC are: Anemia, malaria and thalassemia [7]. RBC count is imperative to verify the immunity and capability of the human body. The anomalous count of RBC can be a sign of disease and the individual may need medical assistance [10][11].

A lot of work has been done to automate the process of RBC counting. Mazalan et. al. [2] compared the two algorithms for counting of blood cells using Hough Transformation and by measuring minimum & maximum radius of blood cells. Patil et. al. [3] proposed a formula for counting of WBC's and RBC's from blood images using gray thresholding. Maitra et. al. [4] talked about different image processing techniques to count the red blood cells using magnification factor and dilution factor. Jambhekar [5] studied about how to classify the different shapes of blood cells using artificial neural network. Mahmood et. al. [6] talked about hough transformation for detecting and extracting the red blood cells based on the geometric features to detect the centre of the circle. Gupta et. al. [7] put forward a technique for automatic segmentation of lesion using adaptive thresholding. Sahastrabuddhe et. al. [8] reviewed different segmentation algorithms and image post-processing techniques like morphological functions, feature extraction and border removing for cell counting based on labeling the connected components.

Thejashwini M et. al. [9] talked about the system which utilize image processing schemes to detect and count the RBC's and WBC's using corresponding digital images. Acharjee et. al. [14] presented a semi-automated image processing system to detect the shape of RBC based on Hough Transformation with the specific diameter. Maji et. al. [15] presented an automated method for counting and characterizing Red Blood cells using mathematical morphology. Gonzalez-Hidalgo et. al. [16] proposed an image processing system for Red Blood cell cluster separation for use in sickle cell disease based on the concept of K-curvature for determining points of interest. Lou et. al. [17] presented a system for counting of red blood cells using different techniques like Hyperspectral image segmentation, Border incomplete cell clearance and classify them using SAM & SVM to generate two dimensional monochrome image. Kolhatkar et. al. [18] surveyed different segmentation techniques for detection and counting of blood cells using image segmentation.

Several other works have also been reported in this area using K-means clustering [19], feature extraction by boundary descriptors [20] and geometric features [21]. Currently, low effectiveness, low speed of cell counting systems, high cost and complexity are the prime shortcomings of the already existing techniques. So there is utmost need to develop a system that can detect and count the Red Blood cells to evaluate the health of the person and to diagnose the diseases like anemia, leukemia etc. with high speed and low cost. In proposed work, some of the technologies that are being used for counting of cells have also been analysed so as to conclude the best appropriate method that can be put into practice to surmount the disadvantages of existing systems.

The major contribution of proposed work is a software system that can automate the manual method of detecting and counting RBCs on the basis of morphological functions using feature extraction, thresholding segmentation and counting of cells using Hough transformation. The most significant and tricky step is segmentation of blood cells because exact cell count is based on the accurate segmentation of blood cells. In addition to that, the uncertainties intrinsic to the microscope image to classify the object as a cell or foreign body, such as dust, can obstruct the image analysis procedure. The proposed algorithm achieves overall accuracy of 91.667% and is computationally very efficient as it takes only 0.81432 seconds to count the number of red blood cells for different blood samples. Low computational time of proposed algorithm makes it pertinent for real time application.

Following parts of this paper are arranged as follows: Section 2 talks about utilized techniques, Section 3 discusses followed methodology, Section 4 states the algorithm proposed, Section 5 demonstrates experimental outcomes and Section 6 gives conclusion.

2. Techniques Utilized

2.1. Image Acquisition

This is the preliminary step of proposed method in which images are segmented and quantised to make them companionable for further processing.

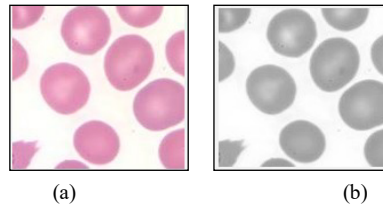


Fig. 1: (a) Input image of blood cells (b) Converted Gray image

Fig.1 shows the input image and corresponding gray scale image.

2.2. Image Segmentation

Image segmentation is a technique used to separate an image into its constituent regions or objects. Segmentation stops when object of interest is isolated.

Segmentation based on thresholding is used to directly produce regions of uniformity within the given image based on intensity values or on some threshold criteria T . In this method, in order to extract objects from background, each pixel in image is replaced by black pixel if the intensity value of image $f(x, y)$ is less than some threshold value T i.e. $f(x, y) \leq T$, or a white pixel if the intensity value of image $f(x, y)$ is more than the threshold value T i.e. $f(x, y) > T$.



Fig. 2: Segmented Input Image

Fig. 2 depicts the segmented image into foreground and background.

2.3. Image Morphology for Feature Extraction

Morphology can be used to extract elements of any image which may be helpful in determining and representing the traits of image regions such as boundary extraction, morphological filtering etc. This prepares image for further processing.

- (a) *Median Filtering*: Median filtering is a non-linear smoothing spatial filter often used for noise reduction, thereby enhancing the quality of the edges in an image. In median filtering, each input pixel is replaced by the median of the gray levels in the m -by- n neighborhood around the corresponding pixels i.e. as given by the following equation:

$$\text{Output pixel} = \text{medfilt2}(\text{image}, [m, n]) \quad (1)$$

where, $[m, n]$ is the size of the mask, 'image' is the input image on which filtering is applied.

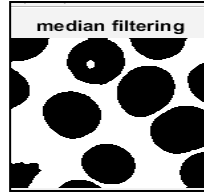


Fig. 3: Median Filtering

Fig. 3 illustrates the image after application of median filtering.

- (b) *Edge Detection*: An edge is a set of connected pixels that lies on the boundary between two regions that differs in value of gray levels. Edges correspond to the discontinuity in the gray level in an image. Sobel edge detection operator is used here which uses a 3×3 mask around each pixel value of an image to approximate the corresponding image gradient. If the image source is defined as A , then the horizontal and vertical derivative approximations are G_x and G_y respectively.

$$G_x = \begin{bmatrix} +1 & 0 & -1 \\ +2 & 0 & -2 \\ +1 & 0 & -1 \end{bmatrix} * A \quad \text{and} \quad G_y = \begin{bmatrix} +1 & +2 & +1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{bmatrix} * A \quad (2)$$

Where, G_x and G_y are the horizontal and vertical derivative approximation and '*' is the convolution operation. Fig. 4 shows edge detection in a sample image.

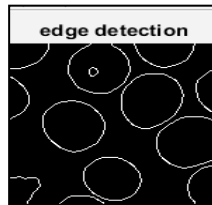


Fig 4: Edge detection

- (c) *Smoothing Using Erosion*: Image smoothing is used to enhance the quality of edges in an image. Region boundaries can be obtained by erosion. The state of a pixel in output image can be determined by applying the imperative in output pixel's value is the least of all the input pixel's neighbourhood. It can be given by the following equation:

$$\text{Erosion} = \{Z / (B) z \subseteq A\} \quad (3)$$

$$\text{Image Boundary} = \text{Original image} - \text{Eroded image} \quad (4)$$

Where, erosion of A by B is the set of all points Z such that B translated by Z is contained in A . Here, set B is the structuring element.

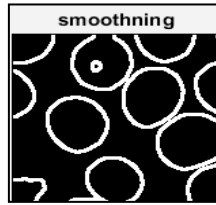


Fig 5: Image Smoothing

In Fig. 5, boundaries of the objects has been obtained by applying erosion.

2.4. Cell counting using Hough Transformation

The most popular method used for counting the number of Red Blood Cells is Hough Transformation technique. As the edge detection using gradient operators like Prewitt, Sobel etc. have some gaps [4]. Red Blood Cells are estimated and detected using this technique by determining the center point of the circle. The circular Hough transform is then applied to detect and draw circles. The circle is described by two following equations:

$$x = a + r \cos\theta \quad (5)$$

$$y = b + r \sin\theta \quad (6)$$

where, a and b are the center of the circle in x and y direction respectively and r is the radius of circle. Parametric form of circle can be given by the following equation:

$$(x - a)^2 + (y - b)^2 = r^2 \quad (7)$$

where a and b are the center of circle in x and y direction respectively.

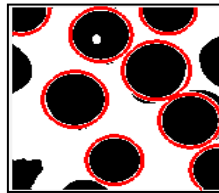


Fig. 6: Boundary detection using Hough Transform

In Fig. 6, boundaries of cells are detected using Hough transformation. Once the boundaries of red blood cells are detected, number of cells can be counted.

3. Methodology

Counting of Red Blood cells through proposed technique is illustrated in Fig. 7. The process comprises of image acquisition followed by numerous image processing operations which are segmentation, median filtering, edge detection, erosion and counting of cells. The counting of Red blood cells will be done on the basis of edge detection using Hough transformation.

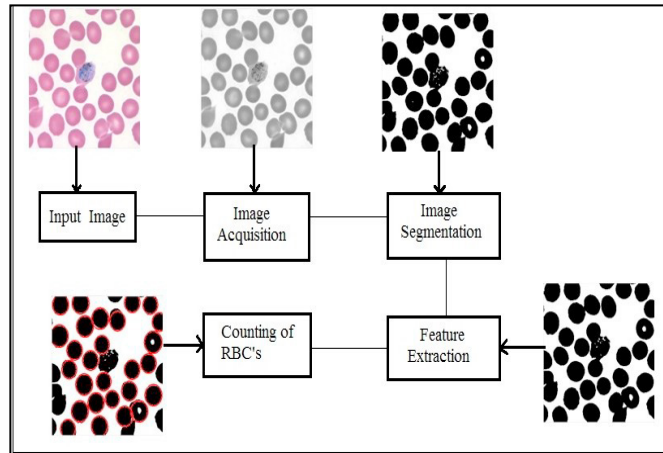


Fig. 7: Proposed Methodology

4. Proposed Method

The proposed method consists of image acquisition followed by pre-processing and thereafter segmentation, feature extraction and counting of blood cells. The entire progression is initialized by acquiring the blood image, which is then transmitted to the further processing level then its thresholding image is generated for applying morphological functions thereafter, counting the total number of red blood cells.

Input: Images of blood cells.

Output: Count of Red Blood cells.

Stepwise details of the proposed algorithm are as under:

Algorithm:

Step 1: Read the image from database. Quantize the image to make it compatible for processing.

Step 2: Segmentation based on thresholding has been used to segment the image in constituent objects based on some threshold criteria T . In this method, in order to extract objects from background each pixel in image is replaced by lowest pixel value if the intensity value of image $f(u, v)$ is less than some threshold value T i.e. $f(u, v) \leq T$, or a highest pixel value if the intensity value of image $f(u, v)$ is more than the threshold value T i.e. $f(u, v) > T$.

$$\text{Thresh} = T[u, v, p(u, v), f(u, v)]$$

$$T(u, v) = \begin{cases} 1, & \text{if } f(u, v) > \text{Thresh} \\ 0, & \text{if } f(u, v) \leq \text{Thresh} \end{cases} \quad (8)$$

Step 3: Various morphological operations are applied on the output image from step 2, viz. median filtering, edge detection and erosion (as discussed in Section 2.3).

Step 4: Total number of red blood cells are calculated using Hough Transform (as discussed in Section 2.4) using following logic.

If <cell detected>
Then

Calculate number of cells
 Else
 Reject.

5. Experimental Results

Experimental outcomes of the proposed scheme are demonstrated in this section. Proposed scheme has been implanted on the Image Processing Toolbox of MATLAB software. Figure 9 shows few of the sample images used for experimental purposes and respective output images with count of red blood cells displayed on the top of the image.

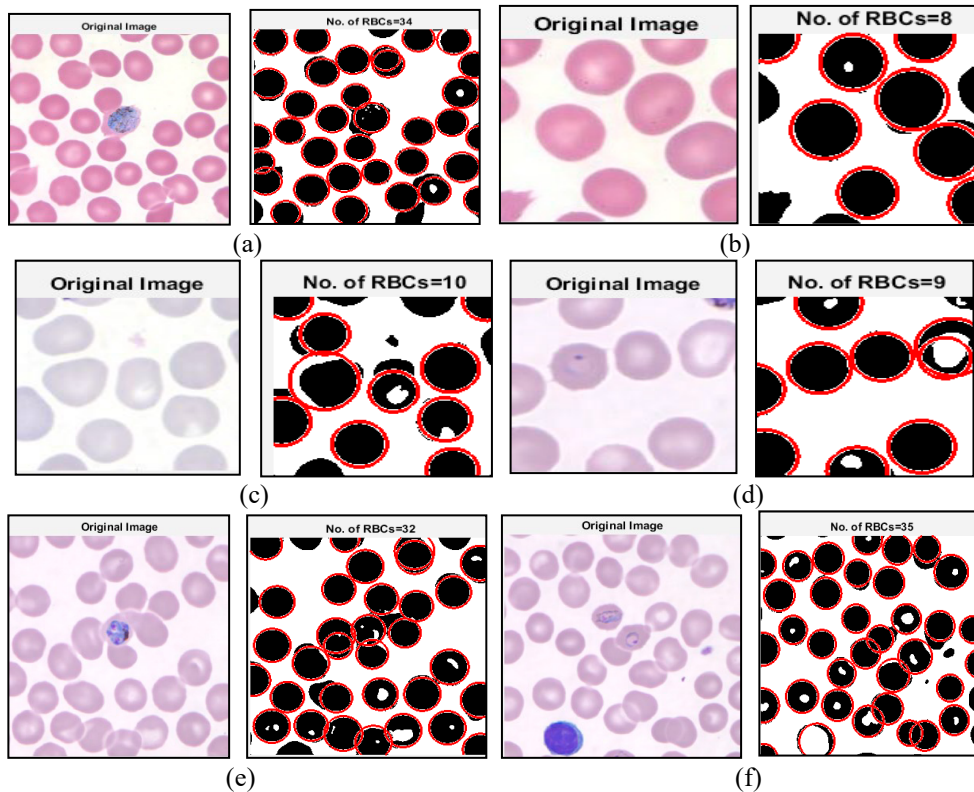


Fig. 8: Few sample images (left) employed in experiments and respective output images (right)

Fig. 8 displays few pair of test images along with respective output images with blood cell count displayed on the top of the image.

Table 1: Experimental results of Joint Image compression and encryption scheme

| Test Images | Manual Counting | Counting by Proposed algorithm |
|-------------|-----------------|--------------------------------|
| Sample 1 | 35 | 34 |
| Sample 2 | 10 | 8 |
| Sample 3 | 12 | 10 |
| Sample 4 | 9 | 9 |
| Sample 5 | 7 | 7 |
| Sample 6 | 10 | 8 |
| Sample 7 | 10 | 9 |
| Sample 8 | 9 | 9 |

| | | |
|-----------|----|----|
| Sample 9 | 33 | 32 |
| Sample 10 | 35 | 35 |

Table 1 depicts the comparison of results between the counting of RBC in images under consideration using proposed algorithm and through manual counting of Red blood cells using hemocytometer through microscopic slides. It clearly shows that proposed approach is able to count the cells with good accuracy.

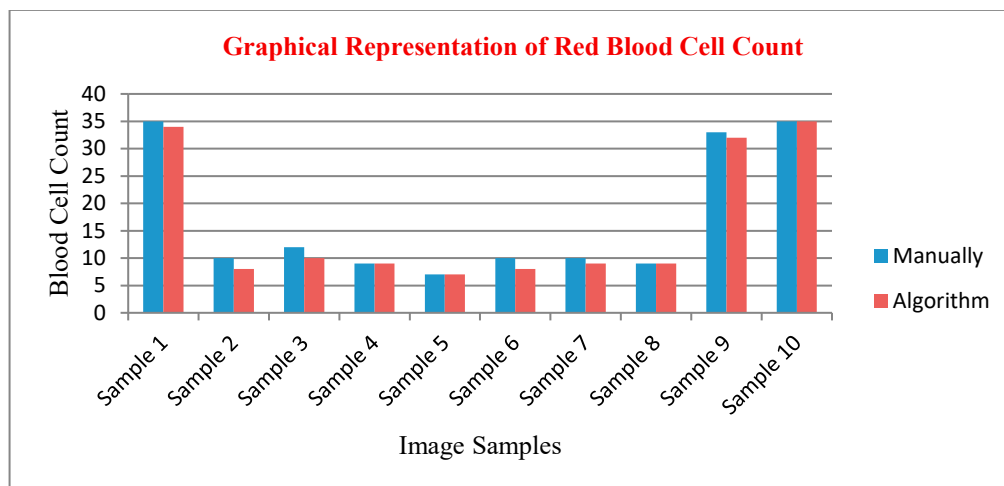


Fig. 9: Graphical representation of Results.

It is observed from the Table 1 that the results obtained from the proposed algorithm offers a good consistency with the manual counting method using microscopic slides. Fig. 9 graphically illustrates the facts presented in Table 1.

Table 2: Computation Time

| Test Images | Computation Time (in seconds) |
|-----------------|-------------------------------|
| Sample Image 1 | 0.9048 |
| Sample Image 2 | 0.7332 |
| Sample Image 3 | 0.7800 |
| Sample Image 4 | 0.7488 |
| Sample Image 5 | 0.7880 |
| Sample Image 6 | 0.7644 |
| Sample Image 7 | 0.7488 |
| Sample Image 8 | 0.7488 |
| Sample Image 9 | 0.9048 |
| Sample Image 10 | 0.9516 |

Table 2 depicts the average computational time for various samples and it also depicts that the proposed scheme is computationally very efficient as it takes only 0.81432 seconds to count the number of red blood cells.

Accuracy

Accuracy is taken as an overall effectiveness of the segmentation technique. It can be calculated using following formula:

$$\text{Accuracy} = (TN+TP) / (TN+TP+FP+FN) * 100 \quad (9)$$

Where, TN and TP are True Negative and True positive respectively i.e. the number of correctly segmented positive

and negative samples. FN and FP are False Negative and False Positive i.e. number of incorrectly segmented samples. Also, to calculate the ability of segmentation sensitivity and specificity factors are calculated using the following [11]:

$$\text{Sensitivity} = TP / (FN+TP) \quad (10)$$

$$\text{Specificity} = TN / (FP+TN) \quad (11)$$

Table 3: Result Division with Frequency

| Images | Image Frequency |
|----------------|-----------------|
| True Positive | 4 |
| True Negative | 7 |
| False Positive | 1 |
| False Negative | 0 |

From the above Table 3 parameters true positive (TP), true negative (TN), false positive (FP), false negative (FN), Accuracy, Specificity and Sensitivity are found

Table 4: Different Parameters

| Parameter | Result |
|-------------|---------|
| Accuracy | 91.667% |
| Specificity | 87.50% |
| Sensitivity | 100% |

Table 4 shows the different parameters like accuracy, sensitivity and specificity for the proposed algorithm. Accuracy is the overall effectiveness of the proposed technique. The proposed algorithm achieves overall accuracy of 91.667% for different blood samples.

6. Conclusion

This paper puts forward an automated process for counting RBCs in acquired images using a number of image processing techniques. The traditional approach of cell count using microscopic slides is based on naked eye observations by technical specialists which is tiresome, prolonged, deliberate and inconsistent. In proposed technique, input data contains images of cells at arbitrary position. Various image processing and morphological operations are applied on the input image for counting RBCs automatically without human intervention. Experimental outcomes show that the proposed technique achieved 91.667% accuracy. This suggests that the proposed technology can be effectively used by pathologists in laboratories to automate the process of cell counting which can be helpful to physician to diagnose disease in swift and proficient way. Future extension of this work may include improving the accuracy of the proposed technique to approach 100%.

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