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ORIGINAL ARTICLE

Identification and red blood cell automated counting from blood smear images using computer-aided system

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Abstract Red blood cell count plays a vital role in identifying the overall health of the patient. Hospitals use the hemocytometer to count the blood cells. Conventional method of placing the smear under microscope and counting the cells manually lead to erroneous results, and medical laboratory technicians are put under stress. A computer-aided system will help to attain precise results in less amount of time. This research work proposes an image-processing technique for counting the number of red blood cells. It aims to examine and process the blood smear image, in order to support the counting of red blood cells and identify the number of normal and abnormal cells in the image automatically. K-medoids algorithm which is robust to external noise is used to extract the WBCs from the image. Granulometric analysis is used to separate the red blood cells from the white blood cells. The red blood cells obtained are counted using the labeling algorithm and circular Hough transform. The radius range for the circle-drawing algorithm is estimated by computing the distance of the pixels from the boundary which automates the entire algorithm. A comparison is done between the counts obtained using the labeling algorithm and circular Hough transform. Results of the work showed that circular Hough transform was more accurate in counting the red blood cells than the labeling algorithm as it was successful in identifying even the overlapping cells. The work also intends to compare the results of cell count done using the proposed methodology and manual approach. The work is designed to address all the drawbacks of the previous research work. The research work can be extended to extract various texture and shape features of abnormal cells identified so that diseases like anemia of inflammation and chronic disease can be detected at the earliest.

Keywords Blood smear · Circular Hough transform · Granulometric analysis · Hemocytometer

1 Introduction

Red blood cells (RBCs), also termed as erythrocytes, are the most common type of blood cell, and the main function is to deliver oxygen to the different body tissues via blood flow through the circulatory system. They carry oxygen from the lungs and release it into tissues by driving through the body's capillaries. The red color of the cells is due to the iron-containing biomolecule termed as hemoglobin. The physiological cell functions like deformability and stability are provided by the proteins and lipids present in the cell membrane. Leukocytes are white blood cells that form the part of the immune system. In the market, hemocytometers are used to calculate the number of red and white cells per liter of blood. It involves the use of physicians' skills to count the cells. It is possible to identify the abnormal cells in hematological conditions, but the features of those cells cannot be calculated. This procedure is often subjected to sampling error which leads to decreased cell count. Automated analyzers like flow cytometry are available in the market to estimate the complete blood count and the leukocyte differential count. The device makes use of a laser beam to identify the cells. The cells are made to pass through the laser beam which makes the cells to reflect back the light which will be detected by the light detector. The chemical and physical characteristics of the cells

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are obtained. It requires small amount of specimen sample and it accurately counts the cells, but it fails to detect the abnormalities in them and is highly expensive. This research work aims to design a system to accurately determine the count of red blood cells and also identify the number of normal and abnormal cells in the image. Analysis of blood smear images plays a vital role in the field of medicine as it helps to diagnose the disease at the early stage. It provides support for the doctors to take the right decision of monitoring and in result, prevention of the disease. The detection of red blood cells is done by initially separating them from the white blood cells. If white blood cells are not separated then misinterpretation of WBCs as RBCs takes place. A counter is set up using labeling algorithm and circular Hough transform. Abnormal cells are detected by taking into consideration a feature termed as form factor which provides details about the irregular dimensions of the cells. A comparison is made between the cell counts done using both the above techniques. We also record the variations between the manual count and count obtained using the proposed approach.

2 Related works

Patil et al. [1] proposed an approach for identification and counting of RBCs. They identified the disadvantages involved in the counting done manually. The dataset had images collected from 15 patients. The input image was converted to gray scale. A median filter was applied to the resulting image. Then, Otsus threshold was applied on the image. The fill operation was performed in order to segment the blood cells properly. The cells at the border were cleared as those would not contribute much to the calculation. The resulting image was labeled with two specific values which were 4 and 8. They obtained an accuracy of 94.58%. They also provided details about the approach followed in the manual count of the RBCs. In this paper, Otsus threshold is used which may produce wrong segmentation if the threshold determined is incorrect.

Alomari et al. [2] proposed an approach for measuring of RBCs using structured cell detection algorithm. The input image was converted to gray scale. A threshold value of 140 was used to obtain the binary image. They found that the threshold value would separate the WBCs from the RBCs. The resulting image was subtracted from the original image to obtain RBCs. The circles were drawn in iterations. The number of iterations were dependent upon the number of edge pixels and distance between edge pixels. The circles detected using this approach were more accurate than those in normal Circular Hough Transform (CHT), but the threshold value of 140 is not suitable for all images. If the WBCs are not accurately obtained, then red blood cells cannot be extracted.

Bhagavathi and Thomas Niba [3] proposed an approach for discovery and enumeration of red blood cells. The input image was applied with adapthisteq function to highlight the red components. The resulting image was used for edge detection. For the purpose of edge detection, the fuzzy rules were framed. An FIS editor was created based on the input values. Rules were set to detect the circles. They assumed that if the radius of the circle fell within the range, then it was identified as pixel 1. If in case the radius fell out of the range, then it was identified as pixel 0. For the detection of the circles with a common center, multiple radiant argument was used. The output of the above operation was an accumulator array with same dimensions as the input image. The resulting image had white background and black boundary. The image was then complemented. Hole-filling operation was performed. Based on the intensities, WBCs were separated from the RBCs. Circle-drawing algorithm was used. The results' section of the paper shows that while applying the circle-drawing algorithm, it also considers the WBCs as RBCs. In order to avoid such errors, a second stage of processing is required to separate the WBCs from the RBCs.

Maitra et al. [4] proposed a methodology for counting of RBC using Hough transform method. The data set had five images. The image was made to undergo a number of steps. The image was converted to gray scale image first. The adapthisteq function was applied to it. The resulting image was given for edge detection. Spatial-smoothing filtering was applied. Hough transform method was used to separate the RBCs from the WBCs based on their size and shapes. After the separation of RBCs from the WBCs, a counter was applied to it. The enumeration of the cells was done by obtaining a formula. Cell count obtained automatically was compared with the ones with manual approach. In this paper, while applying the circle detection algorithm, it detected even the WBCs as RBCs. Hence, a second processing step to separate the RBCs from the WBCs is required.

Mazalan et al. [5] proposed an approach to count the RBC using Hough transform. The input image was cropped in to sub images to obtain radius range of RBCs. The sub image was converted to gray scale. Morphological operations like opening, closing, and reconstruction were applied to it. The resulting image was converted to binary. The border of the image was cleared. The measures like minimum radius, maximum radius, and standard deviation were calculated. Circular Hough transform was used with the radius calculated from the previous step as input.

Venkatalakshmi and Thilagavathi [6] presented Hough transform to support red blood cell counting without manual intervention. The image inputted was mapped to HSV format. The S component of the image was extracted for the further analysis. The image was split into two based on minimum and maximum threshold values determined from histogram. After the application of morphological operations on the images, an XOR operation was performed on the resulting images. The number of circles drawn using CHT (Circular Hough Transform) depicted the number of RBCs.

Sreekumar and Bhattacharya [7] presented circular Hough transform for enumerating the cells. Hue and saturation were eliminated and the contrast of the image was enhanced. Threshold operation was applied to eliminate background from the image. They did not opt for further segmentation as they assumed the red blood cells were circular than white blood cells. Circular Hough transform was applied to detect the red blood cells. The imdistline function was used to obtain the radius range. Approach used was suitable when the white blood cells were deviating from circular shape. Since the WBCs were not separated from the red blood cells, it misinterpreted the WBCs as RBCs. The "imdistline" was suitable when the number of cells were less. The measurement of radius using this function was a tedious task.

Tulsani et al. [8] proposed a method to extract RBCs and count them using watershed transform. The combination of Ycbcr with morphological operations was used to achieve it. In the pre-processing step, the image was freed from the noise, and smoothing was applied. The image was converted to Ycbcr format. Second component of the result was obtained. They identified that Ycbcr format overcame the problem of illumination. Morphological opening with a specific structuring element was used. The resulting image had both WBCs and platelets. Another step of subtraction and opening was performed to obtain two separate images with WBCs and platelets. To obtain only RBCs, the input image was converted to gray scale, and an open by reconstruction operation was used. Erosion and reconstruction operation was applied to the resulting image. The mask of white blood cell was subtracted from the entire image. The overlapping cells were separated from each other using the watershed transform. A final count operation was done to count the cells.

Rakshita and Bhowmik [9] proposed an approach to identify the sickle cells using morphological operations and edge detection. The image was converted to binary, and noise was separated using a Wiener filter. The edge detection was performed using the Sobel operator. Area and perimeter of the cells were estimated to obtain form factor. The objects with form factor in the range (0.5–1) were considered normal, and below were considered abnormal.

3 Problem statement

Determination of red blood cell count plays a vital role in the field of medicine. The present procedures are highly dependent upon the physicians' skills in determining the count. The devices that perform automated counts are highly expensive and they fail in determining the presence of abnormal cells. In order to count the red blood cells in the blood smear, algorithms to accurately extract and count them by separating from other cells are necessitated.

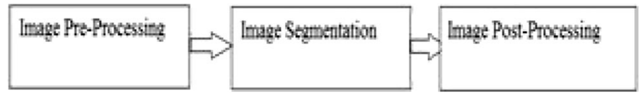

Fig. 1 Stages involved in process of extraction and counting of the red blood cells

4 Methodology

The blood smear images are collected from the Kasturba Medical College, Manipal, Karnataka and Atlas of Hematology. The image is converted to LAB color space as the L layer has luminance close to the human eyes. The white blood cells will have larger size compared to the size of red blood cells. The main aim here is to separate the WBCs from the RBCs. The previous works in the field did not accurately separate the white blood cells from the red blood cells which led to misinterpretation of white blood cells as red blood cells. The K-medoids algorithm is used to segment the image. The mean intensity of image along the red channel is obtained. The WBCs will have minimum intensity. The WBCs are extracted. Morphological operations and modified watershed transform are applied on the image to separate the touching objects. A counter is used to count the cells. The count value is used in later stages to separate them from the red blood cells. Fig. 1 depicts the steps involved in the proposed approach.

4.1 Image pre-processing

(a) Input the image: The blood smear images stained with Wrights stain is given as input to the system. The images are collected from normal patients and cancer patients.
(b) Convert the image to gray scale: The image is converted to gray scale by eliminating the hue and saturation element and retains luminance.

4.2 Image segmentation

(a) Convert the image to binary form: The image is converted to binary using global Otsus threshold as it minimizes the intra-class variance. The level is calculated using this algorithm. It labels objects above the level with one and

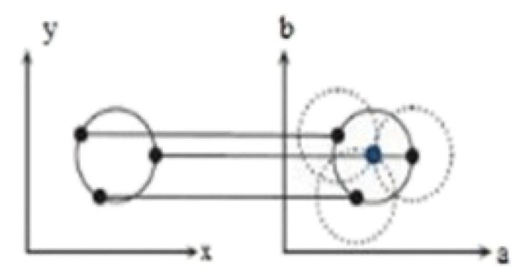

Fig. 2 The points in image space and parameter space with constant radius

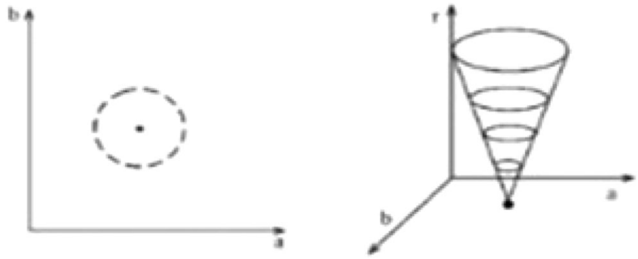

Fig. 3 Parameter space with known radius and unknown radius

other objects with level zero. The holes that cannot be reached from the boundary are filled using the function imfill in Matlab. An erosion operation with a disk-shaped structuring element of size 1 is applied.

(b) Histogram equalization: This research work intends to apply the algorithm to all types of images. Some images require contrast adjustment operation to reduce the disparity. The histogram equalization algorithm enhances the contrast of images by transforming the values in an intensity image so that the histogram of the output image is approximately flat. The resulting image is converted to binary using the global Otsus threshold.

4.3 Image post-processing

(a) Apply watershed transform: Blood smear will have cells overlapping over each other and will be closely attached to each other. Touching cells if not separated generate inaccurate results. The binary image is separated from noise, and distance transform is applied on it. The ridge lines obtained are used to segment the binary image. Tiny local minima are eliminated using the function imextendedmin. Distance transform is then modified so that no minima occur at the filtered out locations using the function imimposemin. The output image will have objects with accurate separation.

(b) Apply morphological open operation: The image is filtered out of the noise using median filter with 2 × 2 neighborhood. The area of all the objects in the image is calculated using region props function and is placed in an array. The array is sorted in descending order so that larger area values are placed in the beginning. An XOR operation with area open function is used to separate the red blood cells from the white blood cells. The function is made to run in a loop. The loop terminates when the WBC cell count reaches zero. The parameters to area open function are lower and upper bound. The lower bound is set as 1, and upper bound is set as the area of the objects. The resulting image will have only the red blood cells. The accuracy of separation was 99%.

(c) Counting of the cells using the labeling algorithm: The objects in the image are labeled using standard connectivity value as 8. The algorithm constructs a matrix with same size as that of the binary image. The background pixels are labeled with a value of zero. Matrix will have non-zero values for the labeled foreground objects. A counter is set to count the number of pixels with label 1. The number of red blood cells was given by the counter value. The abnormal cells are identified using form factor. If the form factor value lies in the range (0.5–1), it is considered as normal cell else they are categorized as abnormal cell. The equation for the calculation of form factor is as follows:

$$Form\ Factor = (4 \times \mathrm{pi} \times Area) \Big/ (Perimeter \times Perimeter) \tag{1}$$

(d) Counting of the cells using circular Hough transform: Circular Hough transform detects the images in a circular

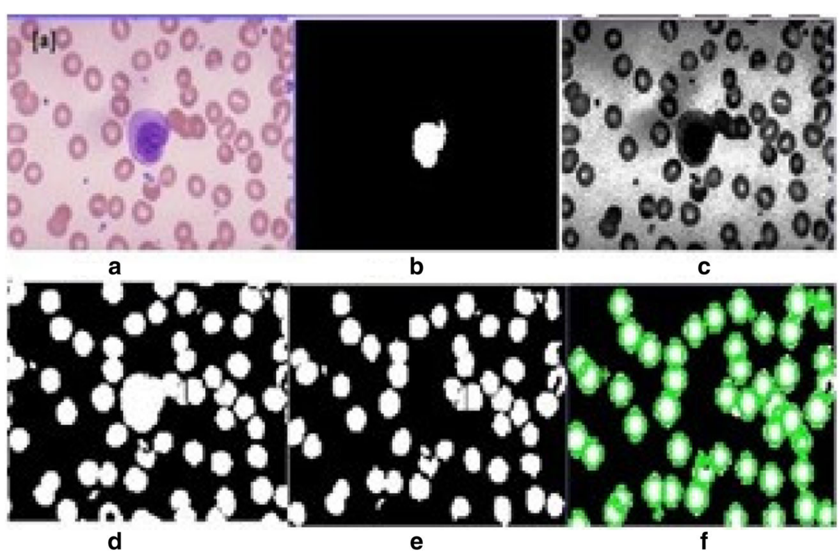

Fig. 4 Red blood cell extraction and counting using labeling algorithm and CHT. (**a**) Represents original image. (**b**) Depicts the white blood cell. (**c**) Histogram-equalized image. (**d**) Depicts the binary image having all the cells. (**e**) Represents only red blood cells. (**f**) Shows the detection of red blood cells using circular Hough transform

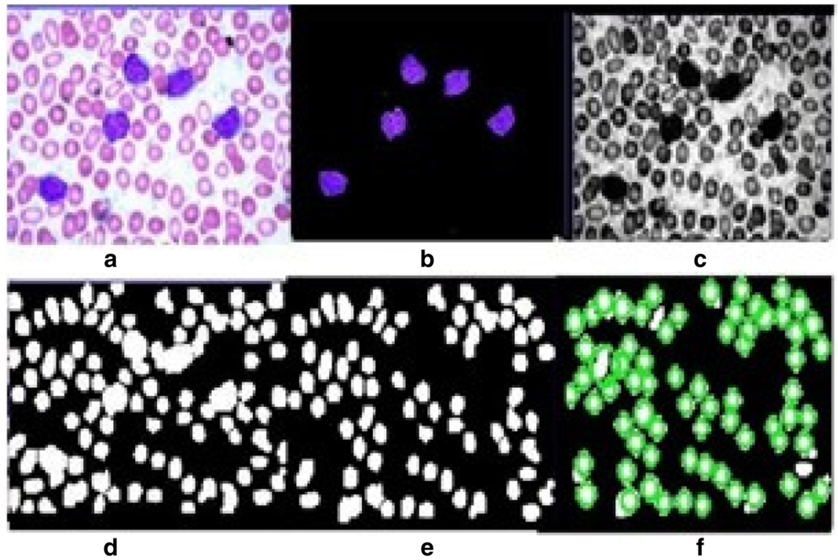

Fig. 5 Red blood cell extraction and counting using labeling algorithm and CHT. White blood cells are accurately separated from the red blood cells

shape. Equation (2) represents the equation of the circle in parametric form. *A* and *b* are the center of the circle in the *x* and *y* direction respectively and *r* represents the radius.

$$(x-a)^2 + (y-b)^2 = c^2 \tag{2}$$

The description of the circle are given in Eq. (3)and Eq. (4).

$$x = a + r\cos(theta) \tag{3}$$

$$y = b + r\sin(theta) \tag{4}$$

There exists many techniques to detect circles in an image. Edge detections like the Prewitt, Canny, and Sobel are used to detect the edges in an image. The circle is drawn at desired radius at each edge point in the parameter space. Fig. 2 represents the parameter space with known radius. The center of the circle is the point at which most of the circles intersect. Circular Hough transform has two cases, one with known radius and the other with unknown radius. When the radius is known, the center values are determined. When the radius is unknown, it becomes a challenging task as there will be three unknowns and the parameter space becomes three dimensional. Fig. 3 depicts the parameter space with known radius and unknown radius.

In this research work, we intend to obtain the minimum and maximum radius by calculating the distances of boundary pixels from the centroid. The procedure is as follows:

(1) The centroid values are obtained for each object in the image.
(2) The distance of boundary pixels from the centroid is calculated.
(3) The maximum and minimum distance values are recorded in an array.

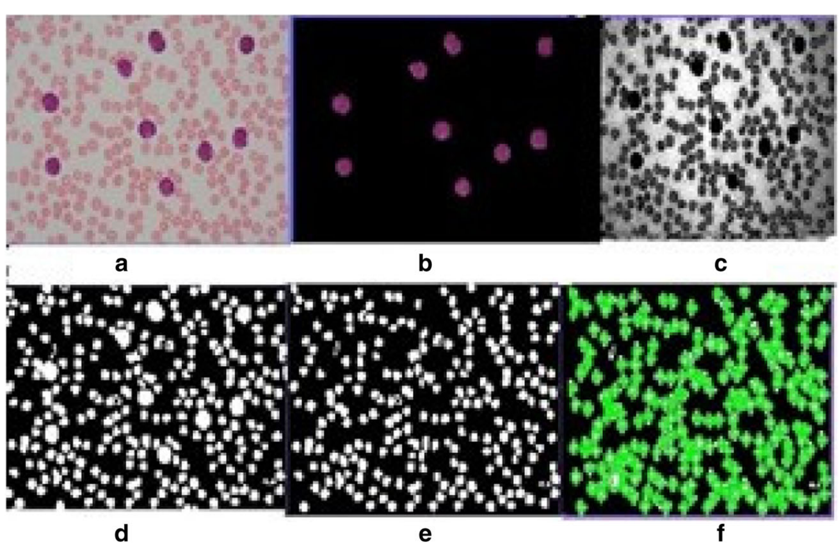

Fig. 6 Red blood cell extraction and counting using labeling algorithm and CHT. (**a**) Represents original image. (**b**) Depicts the White blood cell. (**c**) Histogram-equalized image. (**d**) Depicts the binary image having all the cells. (**e**) Represents only red blood cells. (**f**) Shows the detection of red blood cells using circular Hough transform

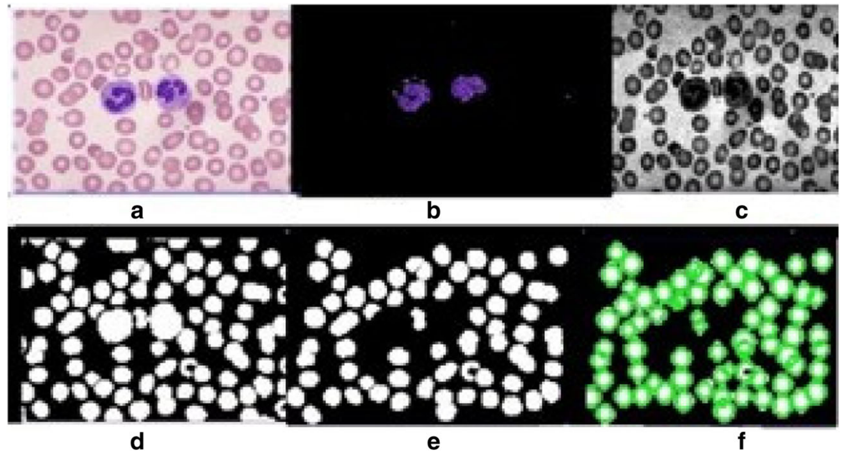

Fig. 7 Red blood cell extraction and counting using labeling algorithm and CHT. (**a**) Represents original image. (**b**) Depicts the white blood cell. (**c**) Histogram-equalized image. (**d**) Depicts the binary image having all the cells. (**e**) Represents only red blood cells. (**f**) Shows the detection of red blood cells using circular Hough transform

(4) The radius range is set for the CHT algorithm by obtaining the maximum and minimum values from the array.
(5) A sensitivity value of 0.76 is given as input to prevent the false positives.
(6) CHT algorithm operates by drawing circles that falls within the specified radius range.

5 Results and discussion

In this section, a comparison is made between the cell count values obtained using the labeling algorithm and circular Hough transform. It is also compared with manual count. K-medoids algorithm is used to obtain WBC mask. The algorithm overcomes the problem of noise which exists in K-means algorithm. Fig. 4 represents the results obtained using the proposed approach. Fig. 5 represents the extraction of WBCs from the blood smear image stained with other than Wrights stain. The contrast of the image is enhanced using the histogram equalization. The RBCs are clearly separated and counted. Fig. 6 represents the image having the red blood cells with very small radius. The automated circular Hough transform accurately counts them by drawing circles with radius computed using distance calculation of the boundary pixels from the centroid. Fig. 7 represents the results obtained using the proposed approach. Fig. 8 depicts the sickle cells extracted from the blood smear of a sickle cell anemia patient. The abnormal cells are identified using the form factor given in Eq. (1). Bounding box is drawn around the abnormal cells which enables the extraction of individual cells for further analysis. The regionprops function is used to draw the bounding box. Texture and shape features of those cells can be calculated that will help in depth diagnosis.

Table 1 shows the comparison between the manual count and count obtained using the proposed technique. A1 depicts the image id. A2 depicts the count obtained using the

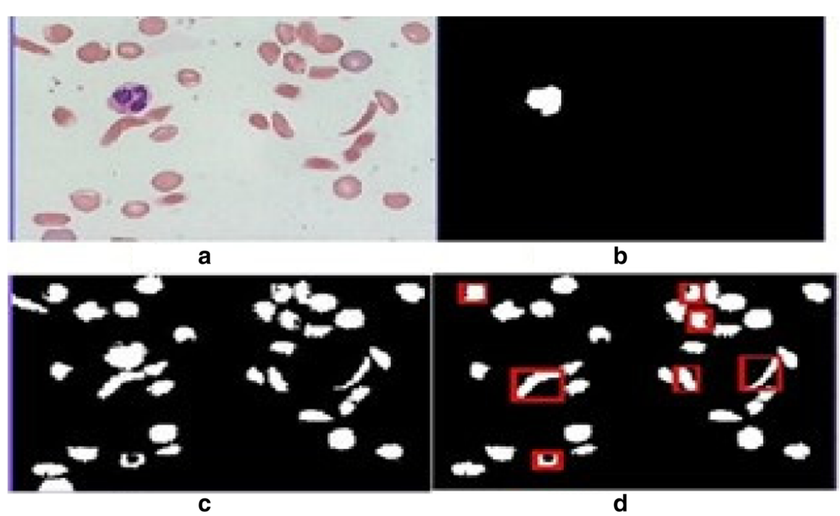

Fig. 8 (**a**) Original image. (**b**) Binary image of the white blood cell extracted using K-medoids algorithm. (**c**) Depicts the binary image having all the cells. (**d**) Depicts the RBCs with bounding box drawn around the abnormal cells

Table 1 Comparison between manual count and count done using proposed technique

A1	A2	A3	A4	A5	A6
1	57	53	40	3	50
2	69	69	70	0	69
3	227	227	210	4	223
4	63	71	50	1	62

automated CHT. A3 shows the count obtained using the labeling algorithm. A4 shows the count obtained using the count obtained by manual counting process. A5 shows the abnormal cell count. A6 represents the normal cell count.

References

1. Patil PR, Sable G, Anandgaonkar G (2014) Counting of WBCs and RBCs from blood images using gray thresholding. Int J Res Eng Technol 3(4):391–395
2. Alomari YM, Abdullah SNHS, Azma RZ, Omar K (2014) Automatic detection and quantification of WBCs and RBCs using iterative structured circle detection algorithm. Comput Math Methods Med 2014:1–17
3. Bhagavathi SL, Thomas Niba S (2016) An automatic system for detecting and counting RBC and WBC using fuzzy logic. ARPN J Eng Appl Sci 11(11):6891–6894
4. Maitra M, Gupta RK, Mukherjee M (2012) Detection and counting of red blood cells in blood cell images using hough transform. Int J Comput Appl 53(16):18–22
5. Mazalan SM, Mahmood NH, Razak MAA (2013) Automated red blood cells counting in peripheral blood smear image using circular Hough transform. International Conference on Artificial Intelligence, Modeling & Simulation: 320-324.
6. Venkatalakshmi B, Thilagavathi K (2013) Automatic red blood cell counting using hough transform. Proceedings of IEEE Conference on Information and Communication Technologies: 268–270
7. Sreekumar A, Bhattacharya A (2014) Identification of sickle cells from microscopic blood smear image using image processing. Int J Emerg Trends Sci Technol 1(5):783-787
8. Tulsani H, Yadav N, Saxena S (2013) Segmentation using morphological watershed transformation for counting blood cells. Int J Comput Appl Inf Technol 2(3):28–36
9. Rakshita P, Bhowmik K (2013) Detection of abnormal findings in human RBC in diagnosing sickle cell anaemia using image processing. In International Conference on Computational Intelligence: Modeling, Techniques and Applications, pp 28–36

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