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CLUSTERED RED BLOOD CELLS SPLITTING VIA BOUNDARY ANALYSIS IN MICROSCOPIC THIN BLOOD SMEAR DIGITAL IMAGES

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ABSTRACT

Clustered Red Blood Cells are observed very frequently in the thin blood smear digital images. Separating clustered Red Blood Cells from the single Red Blood Cells and splitting of clustered Red Blood Cells into single Red Blood Cells is a challenging job in the computer-assisted diagnosis of blood for any disorder in many diseases like Complete Blood Count Test, Anemia, Leukemia and Malaria etc. The mentioned problems are highly laborious in manual microscopy for the hematologists. Many techniques currently existing for the solution suffer from both under- and over- splitting problems when highly complex clusters of Red Blood Cells occur. In addition, the existing techniques are not computationally efficient. In this paper, we address the aforementioned problems, firstly by considering the boundaries of the convex hulls of clustered Red Blood Cells and secondly, by splitting the boundaries according to the number of Red Blood Cells in relation to distance measures. Furthermore, we draw circles using a mid-point circle algorithm at each boundary cleavage to give an illusion of the Red Blood Cells. The test results of the proposed technique on a standard online dataset are presented in two ways. Statistically first of all by achieving an average recall of 0.964 and precision of 0.970 while their F-measure achieved is 0.962 as well as secondly through ground truth data with visual inspections.

Keywords: Automated microscopy; Clustered RBCs; Complete Blood Count; Counting Red Blood Cells; Medical applications

1. INTRODUCTION

In the last decade, automated microscopy has received a great deal of attention from researchers because manual microscopy is still considered as the Gold Standard in the diagnosing of various blood disorders, (Abbas & Mohamad 2013). Due to its ease of availability and low cost, automated microscopy, still preserves the integrity of Gold standard with its light microscopic study capabilities. Despite these factors, it must be recognized that the manual microscopy process requires a high level of expertise and is laborious. In automated microscopy of blood, the main and most challenging job is that of splitting the clustered Red Blood Cells, White Blood Cells etc. The clusters of Red Blood Cells are of two types, i.e. Clumps of Red Blood Cells and Overlaps of Red Blood Cells. The word 'clump' means glue and it is used for the situation in which the Red Blood Cells are glued each other and are formed into long chains. The formation of Red Blood Cells clumps occurs due to an iron deficiency in the blood and

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frequently is observed in diseases like Anemia, Leukemia and Malaria (Abbas & Mohamad 2014a). The degree of severity of these diseases is highly dependent on the number of Red Blood Cells, e.g. in Malaria the Parasitemia is the ratio of infected Red Blood Cells to all Red Blood Cells observed on the slide. In automated diagnosing, the accuracy of diseases, in which the counting of Red Blood Cells is involved, is highly affected by clusters of Red Blood Cells due to the consideration of a cluster as single object, while in reality there is more than one cluster. In addition, important information is hidden in these clusters (Saadi et al., 2014). Overlapped Red Blood Cells are few in number and their formation is often due to improper slide preparation. Both of these problems affect the counting accuracy in manual as well as automated microscopy, while their cleavage in a proper, easy and computationally less expensive way is required.

Recently, extensive efforts have been made by researchers to develop algorithms for splitting the clumped and overlapped Red Blood Cells, i.e. clustered Red Blood Cells. The developed techniques show a considerable degree of success, but still there is space for improvement. The approaches adopted by previous studies to combat the problems are divided into the following categories, i.e. Morphological operations including erosion, dilation or opening and closing to split the clusters of Red Blood Cells (Buggenthin et al., 2013; Prasad et al., 2012; Amit Kumar et al., 2012). However, the main problem in a morphological-based approach is that it works well in overlapping Red Blood Cells, but the overlap will not be more than two cells, but in reality we have some clumped RBCs, which are very long chains.

Concavity-based approaches deal the problems in such a way so as to discover concavity regions and in some cases to find concavity points and to split the clustered Red Blood Cells through linear cuts or circular drawing or elliptical drawing, as stated in the studies of (LaTorre et al., 2013; Tafavogh et al., 2013; Zhang et al., 2012; Wang et al., 2011; Kumarasamy et al., 2011; Wen et al., 2009; Makkapati & Naik 2009; Gurcan et al., 2009; Cloppet & Boucher, 2008; Abbas & Mohamad, 2014b; Abbas & Mohamad, 2015c). The concavity-based approaches give good results, but in some cases, they are computationally very expensive.

Watershed-based techniques include all forms of watershed-based algorithm, etc., (Tulsani, 2013; Ferro et al., 2013; Hodneland et al., 2013; Schmitt & Reetz, 2009; Schmitt & Hasse, 2009; Špringl, 2009; Abbas & Mohamad, 2015a). Watershed-based approaches have a certain degree of success, but in dense clumps they resulted in over-segmentation, while in some cases, they also suffered from the problem of under-segmentation.

Edges or contour-based techniques can offer solutions in the form of analyzing split edges and linkages of contours, etc. (Gonçalves & Bruno, 2012; Kong et al., 2011a; Kong et al., 2011b). This approaches works well, but it requires models, based on some templates and they are complex, both in execution as well as in implementation. A model-based approach offers various models in the form of circles through various theories like Gestalt or geometrical theories, etc., (Köppen et al., 2007; Jiang et al., 2006; Airsang et al., 2013). The problem in this approach is that it seems to be unrealistic, due to its highly complex nature and implementation. In addition, contour-based techniques are computationally too much expensive.

Some studies do not consider splitting the clustered Red Blood Cells, but they rely on an area-based estimation approach (Owais Shaikh et al., 2013-2014; Nguyen et al., 2011). The problem in this approach is that we need separation or splitting of RBCs because when they are in cluster they hide important information. Moreover, we cannot rely on the area of the RBCs because the area of RBC varies from patient to patient and can be disturbed even on the slide.

The Circular Hough Transform (OHT)-based approach mainly considers the Red Blood Cells as being circular in shape. This consideration is not correct because of highly alterable RBC's morphology, e.g. tear drop, holly jolly, moon like RBCs, etc. (Mahmood et al., 2013; grietinfo.in, 2013; Mahmood & Mansor, 2012; Ramesh et al., 2012) Some studies achieved a high degree of accuracy, but involuted human intervention means semi-automatic behavior (Abbas et al., 2014; Abbas et al., 2015).

2. METHODOLOGY

The proposed methodology for splitting the clustered Red Blood Cells is implemented in the experiments of the proposed algorithm that are carried out on the standard image dataset obtained from (DPDx 2002). The proposed method is promising in that it is computationally efficient. In this study, we considered the universal property of the boundary condition of the Red Blood Cells because the rest of the properties like colour, shape and other attributes are highly changeable, due to some other kind of disorder that may vary from patient to patient. The study starts with the separation of single and clustered Red Blood Cells (Clumped and Overlapped) through a double check mechanism. This separation has been made for the purpose of trimming the processing time. In the first method, we calculate the area and the number of pixels defining an object for each Red Blood Cell. Then, we calculate the median area. The cause behind the consideration of the median value among many other central tendency measures is that when the data values contain small and large values irregularly, then median value is the best option. When we divide the area of every Red Blood Cell with the median area, the result obtained, if it equals to 1 or near to 1, it is regarded as being a single Red Blood Cell and it is considered for a mask of single Red Blood Cells, while the negation results in a multi-Red Blood Cells mask. Then, we pass the single RBC mask to the pixel index list (IDX) of the input image to obtain the image of single Red Blood Cells, while in passing over the multi-mask; we obtained the image of clustered Red Blood Cells. In the second method, we calculated the median of the elongation by using Equation 1 (Abbas & Mohamad, 2015b).

$$Elongation = \frac{Length}{Breadth} \tag{1}$$

where *Length*=Major Axis, and *Breadth*= Minor Axis.

The median value here is based on elongation instead of the area, while the rest of the whole procedure is same as mentioned above (Abbas & Mohamad, 2015b). The simulated result of the concept is given in Figure 1.

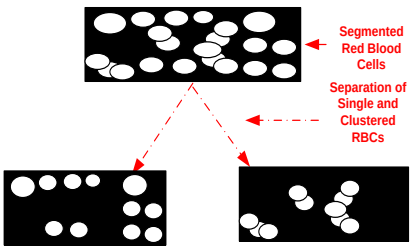


Figure 1 The simulated results showing the concept of separation of single Red Blood Cells and clustered Red Blood Cells

In Figure 1, the top image is the binary form of the input image, obtained through automatic thresholding (ATF), while the holes are filled and small areas are removed as noise (Rahman et

al., 2013). Thus, after applying the algorithm as presented as 2.1, this action resulted in the form of two separated images, one having single Red Blood Cells, while the other has clustered Red Blood Cells. Next, we presented the algorithm for separation of single Red Blood Cells and clustered Red Blood Cells in the below given algorithm, while in comparison the overall methodology of this study is depicted in Figure 2.

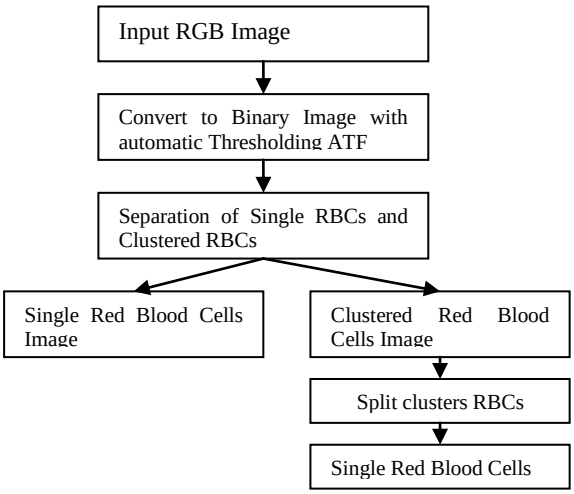
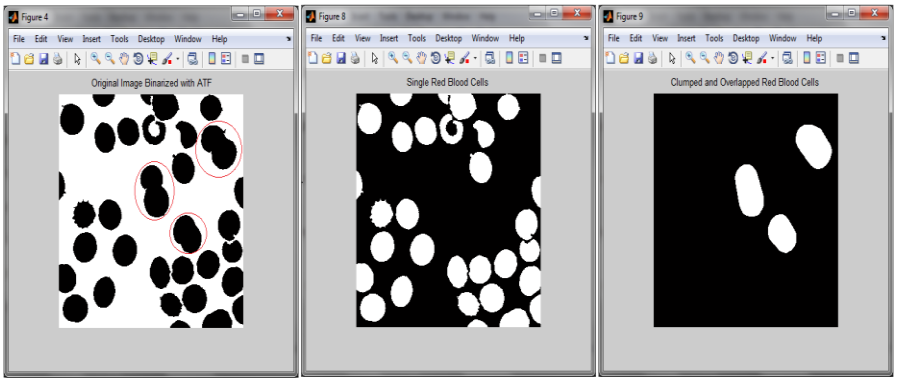


Figure 2 Overall methodology of this study

2.1. Separation of Single and Clustered RBCs Algorithm

Arguments: Original Binary Image 'OBW'
Return: Single RBCs Image, Clustered RBCs Image and MCcount the number of Red Blood Cells in each cluster

1. *Find the Convex Hull of each object in the OBW as COBW*
2. *Find Area and Elongation of each object in COBW*
3. *Calculate area of each object in COBW*
4. *Calculate Major axis of each object in COBW*
5. *Calculate Minor axis of each object in COBW*
6. *Elongation= MaArc / MiArc*
7. *Median of Area of RBCs*
8. *Median of Elongation of RBCs*
9. *Find Out the Single RBCs Masks*
10. *Clustered RBCs Mask = Negation of Single RBCs Mask*
11. *Counting the number of RBCs in each Cluster through division of each RBCs area by Median area and Median Elongation*
12. Single RBCs Image pass Single Mask to PixelIdxList
13. Clustered RBCs image pass clustered Mask to PixelIdxList



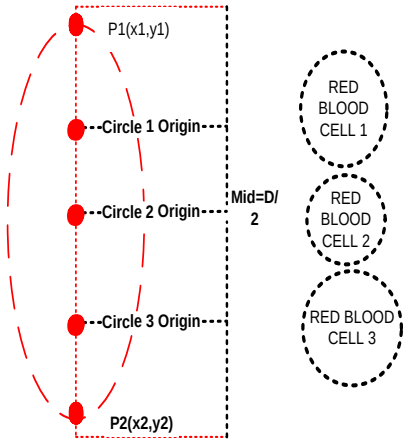
a) Input Image b) Single RBC c) Clustered RBCs

Figure 3 Separation of Single and Clustered RBCs

In Figure 3, Matlab results of the algorithm are presented. Image a) is the original binary image binarized with automatic thresholding while the result of Image b) is a single RBC image and Image c) contains the clustered RBCs as we took the convex hulls of the clustered RBCs for increasing accuracy.

2.2. The Splitting Process of the Clustered Red Blood Cells

After separation of single and clustered Red Blood Cells, the actual work of splitting the clustered Red Blood Cells starts. In this regard, we adopted the approach of boundary analysis. For increasing accuracy, we considered the convex hulls of the clustered RBCs and we calculated the convex hulls of all the clustered RBCs to determine whether, it is clumped or overlapped with Equation 2 and the conceptual design of this approach is presented in the simulated diagram depicted in Figure 4.



Distance D between P1 and P2 Center = D/2
P1= Starting Point of the Boundary
P2= Length(Boundary)/2
Number of Circles =Boundary distance/No.of RBCs

Figure 4 The simulated diagram presenting the concept of how to analyze the boundary of convex hull and the split clustered RBCs

$$\sum_{i=1}^{|X|} \alpha_i x_i \mid (\forall_i : \alpha_i \geq 0) \,\&\, \sum_{i=1}^{|X|} \alpha_i = 1 \qquad (2)$$

where, |X| = finite set of points, x_i is point |X| while α_i is weight assigned to x_i, the sum of the weights must be equal to 1 mean normalized. After finding the convex hulls, we determined the boundaries of every clustered RBC as shown in Figure 4. We divided the boundaries of every clustered RBC into two halves using Equatoin 3 and we measured the distance between point P1 and the mid-point P2 with Equation 4.

$$Index = \frac{Length(Boundary)}{2} \tag{3}$$

where the boundary is the boundary of clumped or overlapped RBCs and the index is the index of boundary containing its points.

$$D = \sqrt{\frac{(x_2 - x_1)^2 + (y_2 - y_1)^2}{2}} \tag{4}$$

$$No.of\ .Parts = \frac{D}{No.ofRBCs} \tag{5}$$

where, the number of RBCs we can determine by dividing the convex hull area by the median area of a single RBC. After division of the boundary area into parts, then we draw a circle by finding a mid-point in between two consecutive points and using the mid-point circle algorithm with 4-way symmetry to draw circles separated from each other.

2.3. Splitting of Clustered Cells through the Boundary Analysis and Circle Drawing Algorithm

Arguments: Binary Image 'BW' having Clumped and Overlapped RBCs, MCcount is an array containing the number of RBCs in each cluster

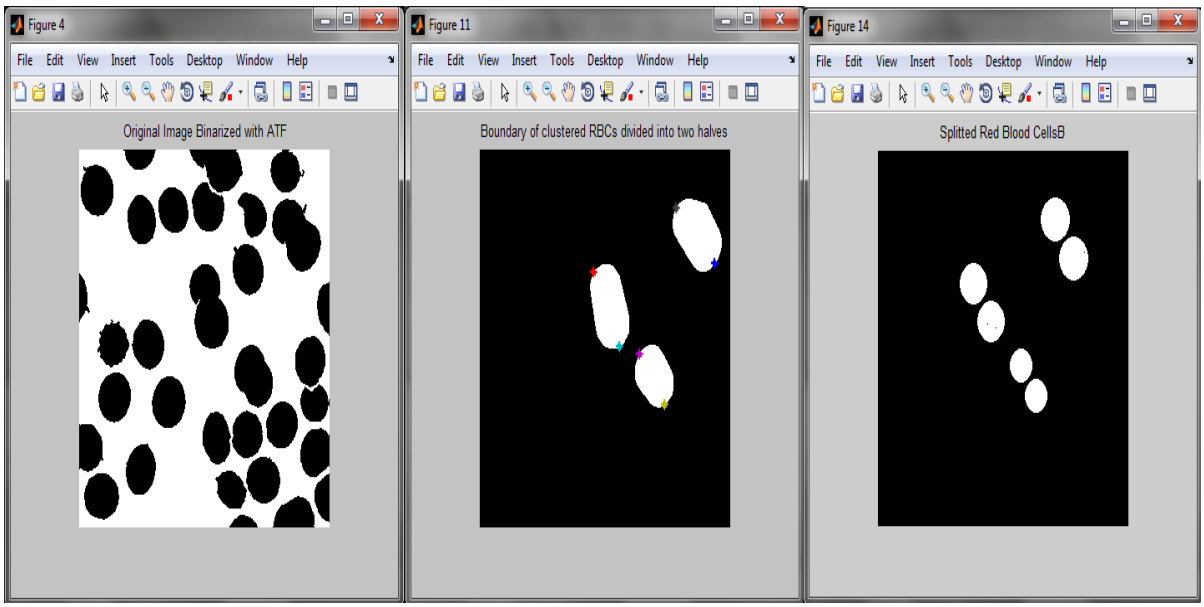
Return: Binary image of Split Single RBCs (BWS)

1. *Find the Convex Hull using Equation 3.1 as BWC*
2. *Empty image same as BWC*
3. *Trace boundaries of each cluster in BWC;*
4. *Assign the boundary of each object*
5. *Number of RBCs in each cluster as nfc*
6. *Find the kth boundary in B*
7. *Calculate the mid -Boundary using Equation 3.2*
8. *Points P1 as the First point of Boundary and P2 as Mid-point of Boundary*
9. *Find the Distance between P1 and P2 using Equation 3.3*
10. *Division of clumps into parts*
11. *Part ← rounded D/nfc(k)*
12. *Move P2 to Part location*
13. *MidPart ← Part/2*
14. *Emt ← Emt+ Call Mid-Point Circle*
15. *for i ← 2 up to number of cells in cluster k*
16. *Move P1 to P2*
17. *Emt ← Emt+ Call Mid-Point Circle*

End for

18. *Reset P1 and P2 to zero*
19. *Filled the Split Single RBCs*
20. *BWS ← Filled Emt*

Return BWS.



a) Original Image b) Input Image c) Output Image

Figure 5 Presents the Matlab results of the Splitting Algorithm as mentioned above

In Figure 5, Image a) Presents the original binary image having clustered RBCs; Image b) Presents the Convex hulls of the clustered RBCs and also highlights the Points P1 and P2 while further division is based on the number of RBCs in the cluster and the distance calculated between P1 and P2. Finally, Image c) presents the circles and split-clustered Red Blood Cells.

3. RESULTS AND DISCUSSION

We performed all the experiments on microscopic thin blood smear digital images obtained from (DPDx, 2002). The results in this section are presented qualitatively and as well as quantitatively.

3.1. Qualitative Analysis of the Results

The first method presents the results on a qualitative basis through ground truth images and these can be verified with visual inspection.

3.2. Quantitative Analysis of the Results

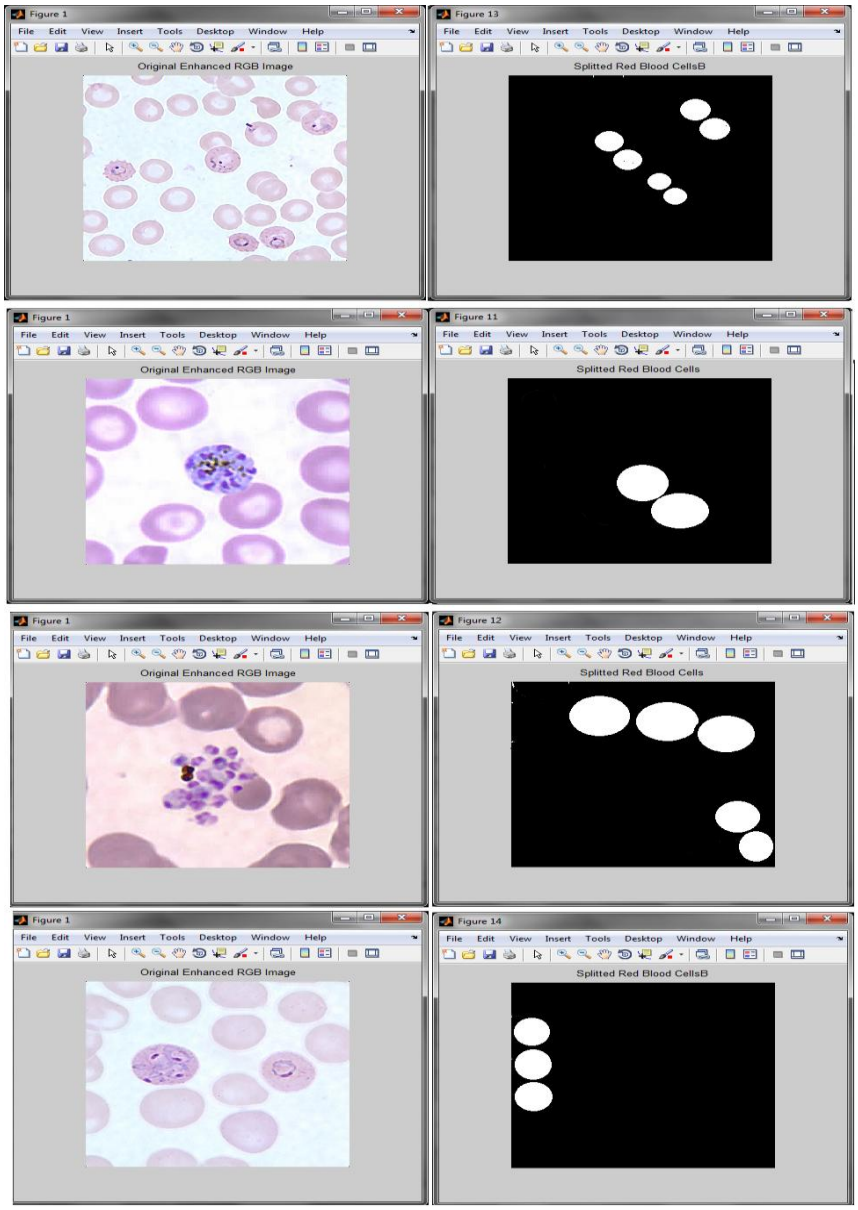
In this category, we analyzed the results by performing experimentation on a set of 20 images. We calculated the precision and recall of each slide and then found an average precision and recall for calculating the average F-measure (Reis et al., 2014).

$$\Pr ecision = \frac{|Tp|}{|Tp + Fp|} \tag{6}$$

$$\text{Re}call = \frac{|Tp|}{|TP + Fn|} \tag{7}$$

$$F - measure = 2 \times \frac{\text{Pr}ecision \times \text{Re}call}{\text{Pr}ecision + \text{Re}call} \tag{8}$$

The equations to calculate precision, recall and the average F-measure are presented as Items 6, 7 and 8 respectively where Tp= Number of correctly counted RBC, Fp= No. of RBCs Incorrectly counted Fn= Number of RBCs incorrectly leftover from counting. In addition, the F-measure is the harmonic mean of precision and recall used to find accuracy.



Column (A) Column (B)

Figure 6 Column (A) presents Input Microscopic thin blood smears digital Original Images and Column (B) presents Output images as a result of the proposed method

Table 1 Quantitative Analysis of the 20 images dataset obtained from (DPDx, 2002)

Slide No.	Total Number of RBCs including clusters	Total number of RBCs with counting exact no. of RBCs in each cluster through Visual inspection by experts	Total number of no. of RBCs counted through computer after splitting the clustered RBCs	Precision	Recall	F-measure
1.	33	37	36	1	0.97297297	0.986301
2.	45	55	52	1	0.90909091	0.952381
3.	41	47	43	0.95918367	1	0.979167
4.	59	63	63	1	1	1
5.	42	55	55	0.93220339	1	0.964912
6.	30	45	43	0.9375	1	0.967742
7.	08	20	18	1	0.9	0.947368
8.	98	105	99	0.95454545	1	0.976744
9.	32	35	33	1	0.94285714	0.970588
10.	22	26	26	1	1	1
11.	12	19	19	0.9047619	1	0.95
12.	17	23	21	1	0.91304348	0.954545
13.	19	29	23	1	0.79310345	0.884615
14.	58	59	55	0.93650794	1	0.967213
15.	15	20	17	0.83333333	1	0.909091
16.	44	51	46	1	0.90196078	0.948454
17.	39	40	39	1	0.975	0.987342
18.	24	29	27	0.90625	1	0.95082
19.	88	97	94	0.93269231	1	0.965174
20.	72	81	78	1	0.96296296	0.981132

The results presented in Table 1 include normal (black fore-colour) and abnormal (red fore-colour) Red Blood Cells slides. The proposed technique achieved an overall average precision of 0.964, which shows the correct counting accuracy by examining correct identification of RBCs and leaving out incorrect identification in counting. The average recall achieved by the proposed method is 0.964; it shows the correct counting accuracy by examining the correct rejection of RBCs and leaving the incorrect rejection of RBCs in counting. The overall average F1-score or F-measure of precisions and recall achieved is 0.962, which shows a high degree of accuracy in the field.

4. CONCLUSION

The main aim of this paper is to achieve high accuracy in the counting process of Red Blood Cells by splitting the clustered (Clumped and overlapped) Red Blood Cells, achieved by examining the boundaries and distance for splitting. The number of Red Blood Cells depends on the mask by performing a double check and examining the area of the convex hulls of the Red Blood Cells. The overall harmonic mean of the precision and recall achieved is 96%, which shows the accuracy of the proposed method. However, the method needs improvement for smoothing the boundaries, as on smoothed boundaries the distance will be calculated more accurately.

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