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OCCLUDED RED BLOOD CELLS SPLITTING VIA BOUNDARIES ANALYSIS AND LINES DRAWING IN MICROSCOPIC THIN BLOOD SMEAR DIGITAL IMAGES

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ABSTRACT. *Occluded Red Blood Cells are observed frequently in the thin blood smear digital images. Separating the occluded Red Blood Cells from the single Red Blood Cells and further the splitting of occluded 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 problem is also highly laborious in manual microscopy for the hematologist. Many techniques currently existed for the solution but suffered from both under and over splitting problems when highly complex occlusions of Red Blood Cells occurred. Also the existed techniques are not computationally efficient. In this, paper we address the mentioned problems in realistic, efficient and automated way by considering the boundaries of the occluded Red Blood Cells through convex hulls to divide the boundaries on the basis of distance calculation in a very simple and efficient way split the occlusions according to the number of Red Blood Cells. Further, we draw lines using Digital differential analyzer graphics line drawing algorithm at the two respective end points to give cuts to split the occluded Red Blood Cell. The test results of the proposed technique using standard online data set of thin blood smear digital images (provided by Centers of diseases Control and prevention, USA) are presented in this paper by qualitatively analysis through ground truth with visual inspection.*

***Keywords**:* Occluded RBCs, Thin Blood Smears, Anemia, Concavity Regions, Automatic diagnosing, convex hulls, Concavity Points.

1. **Introduction.** In the last decade, automated microscopy has got much attention of the researchers for the purposes that the manual microscopy is still considered as a Gold Standard in the diagnosing of various disorders in blood. Due to ease of availability and low cost still preserve the right of Gold standard with manual microscopy but it must also be not ignorable that the process requires high expertise and is too laborious. In automated microscopy of blood the main and most challenging job is that of occluded Red Blood Cells, White Blood Cells etc. The occlusions of Red Blood Cells are further divided by this study into two types i.e. Clumps of Red Blood Cells and Overlaps of Red Blood Cells. The word clump means glue and is used for the situation in which the Red Blood Cells glued each other and formed long chains. The formation of clumps of Red Blood

Cells occurs due to iron deficiency in blood and is the frequent observed in diseases like Anaemia, Leukaemia, Malaria and many other diseases. The degree of severance of these diseases highly dependant 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 counting of Red Blood Cells is involved are highly affected by clumps of Red Blood Cells due to the consideration of a clump as one single object while a single clump may encompasses more than six Red Blood Cells. Also important information is hidden in these clumps. On the other hand overlapped Red Blood Cells are few in numbers not more then four Red Blood Cells combination and formation is just 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 the need of the day.

2. **Literature Review.** Recently, too many efforts have been made by researchers to develop algorithms for splitting the clumped and overlapped Red Blood Cells i.e. the clustered Red Blood Cells and show a high degree of success but still there are gaps which are not addressed or addressed in computationally expensive and some cases impossible ways. The approaches adopted by previous studies to combat the problems are divided into the following categories i.e. Morphological operation based includes erosion, dilation or opening closing to split the clusters of Red Blood Cells [1],[2],[3]. However, the main problem in morphological based approach is that it works well in overlap of Red Blood Cells not more then two cells but in reality we have some clumps which are very long chains. Concavity based approaches deal the problems in the way to find out the concavity regions and some cases the concavity points and split the clustered Red Blood Cells through lines cuts or circles drawing or ellipses drawing as stated in the studies of [4], [5], [6], [7], [8], [9], [10], [11] and [12]. The concavity based approaches gives good results but in some cases they are computationally very expensive. Watershed based techniques includes all form of watershed algorithm based etc as presented by the studies of [13], [14], [15], [14], [16], [17] and [18]. Watershed based approach have certain degree of success but in dense clumps it results in over segmentation while in some cases also suffered from the problem of under segmentation. Edges or contour based techniques can gives solution in the form of analysing split edges and linkages of contours etc as mentioned in the works of [19], [20] and [21]. This approach working well but required model based on some templates and burden some both in execution as well as in implementation. Model based approach gives various models in the form of circles through various theories like Gestalt, geometrical theories etc as presented in the work of [22], [23] and [24]. The problem in this approach seems to be unrealistic as due to its highly complex nature and implementation. Also it is computationally too much expensive. While some studies do not consider the clumps and overlaps of Red Blood Cells for splitting but they relay on guessing Area based estimation approaches as mentioned in the work of [25] and [26]. The problem in this approach is that in some cases we want to note the disorder as well in the Red Blood Cell in such case this approach will fails while also the areas of Red Blood Cells by most of the studies considered as circular, which is not true as because morphology of the Red Blood Cells highly changes due to any disorder. Circular Hough Transform based approaches as mentioned by [27], [28], [29] and [30] mainly considered the Red Blood Cells as circles which is not true as mentioned above.

3. **Proposed Methodology.** In this study we first separate the single Red Blood Cells from occluded Red Blood Cells for the purpose of efficiency. Separation is considered by this study on the basis of double check due to the varaying sizes of Red Blood Cells. The whole idea of this study is depicted in the flow diagram Figure 1while the simulated image of the whole process is presented as Figure 2. The original input image is converted to binary by considering histogram and on the basis of the histogram an appropriate value for thresholding is calculated, the values less then the threshold value is replaced as 0s while the greater values are replaced with 1s. After this small areas are removed as noise while holes in the RBCs are filled. The formations of holes in the RBCs are due to the similarity of the center of the RBCs with the background and because of hemoglobin as RBCs have no nucleus. Next, we find out the convex hulls of all the Red Blood Cells through Equation mentioned as 1, for the purposes to increase the accuracy. The separation of the single and occluded Red Blood Cells has been made using two methods to increase the accuracy and enhance consistency. The first method is the comparison of areas (Number of pixels defining the object) through a median central tendency measure, all the areas of the RBCs are divided by the median value and the results are combined in array as values near to or one are considered as single RBCs while values results greater are considered as occluded RBCs, next these masks are passed to the pixels IDX list of the original image for the purpose to obtain two images one as single having non-occluded RBCs image while the other as occluded

Red Blood Cells having only occluded Red Blood Cells. In the same way, for the second check we consider Elongation instead of Area through Equation presented as 2, while the rest of the process is the same. We performed the double check because of variation in the sizes of Red Blood Cells the area is not enough to take decision.

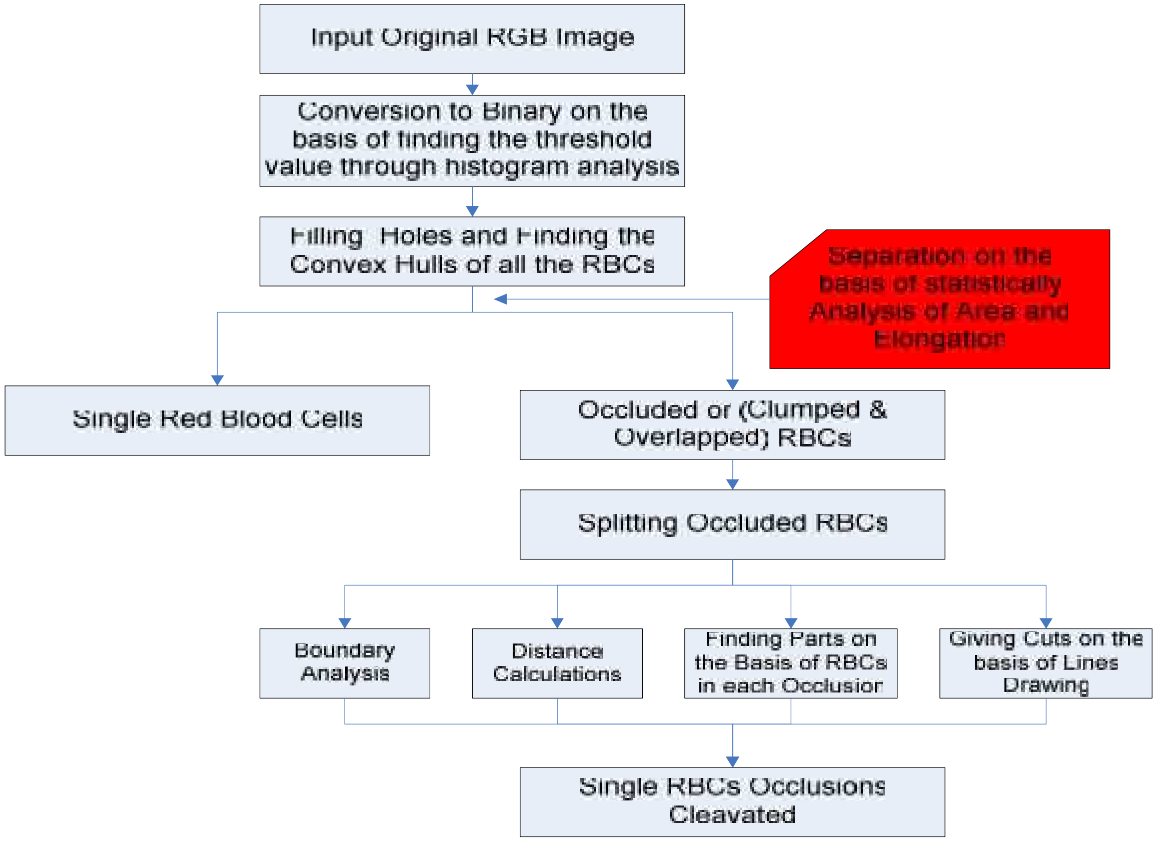

Figure 1Diagramatic Representation of the overall Research Methodology

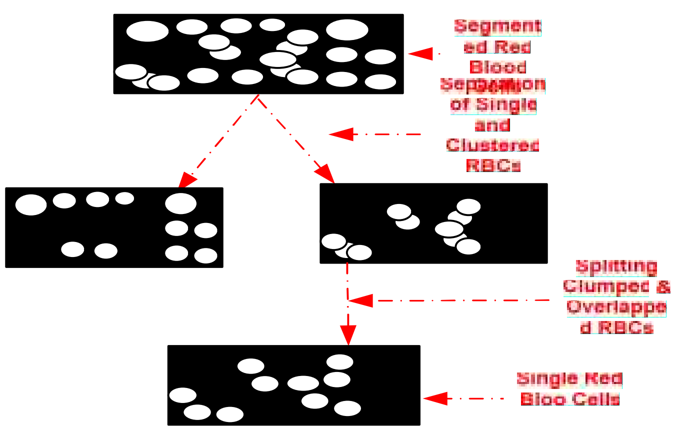

Figure 2 The simulated diagrammatic reprsentation of the whole process

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

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.

$$Elongation = \frac{Length}{Breadth} \qquad (2)$$

where, Length=Major Axis while Breadth= Minor Axis

After separation of single and occluded RBCs, the next process is that of splitting the occlusions (clumped and overlapped) of Red Blood Cells. In the splitting process we first trace the boundaries of all occluded Red Blood Cells, first we divide the boundary into halves using equation 3, then taking the first point of the boundary as P1(x1,y1) while, P2(x2,y2) is the last point of the first half of the boundary. After, finding the points P1 and P2 we calculate the distance between P1 and P2 using equation 4, once find out the distance the next process is to divide the boundary according to the number of Red Blood Cells in each occlusion and take these division points and the distances between the consecutive points using equation 5. The same points are marked on the other half of boundary and then using Digital Differential Analyzer graphics algorithm to draw lines in between respective end points and this way after applying a slight erosion(Morphological operator) the occlusions are cleaved into single Red Blood Cells. The idea is simulated in the diagram depicted as Figure 3.

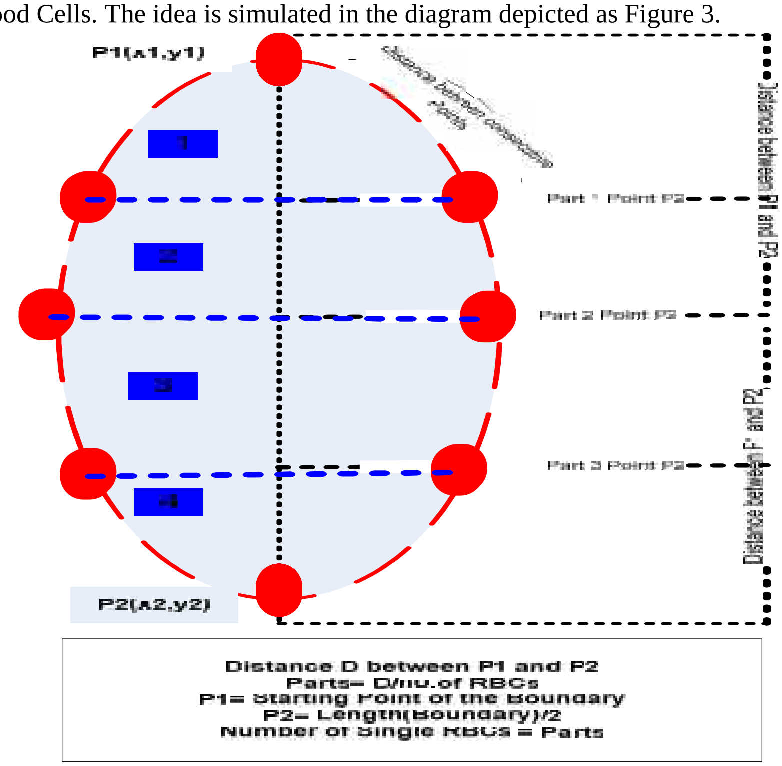

Figure 3 The simulated diagram of the concept of splitting

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

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

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

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

where, Number of RBCs we can found while dividing the convex hull area by the median area of single RBC.

4. **Results.** In this sect we are presenting the results obtained from the implementation of the above concepts through visual inspection with ground truth marked by experts. The experimentation has been carried out on 20 images dataset obtained from DPDx [31].

First Input Image

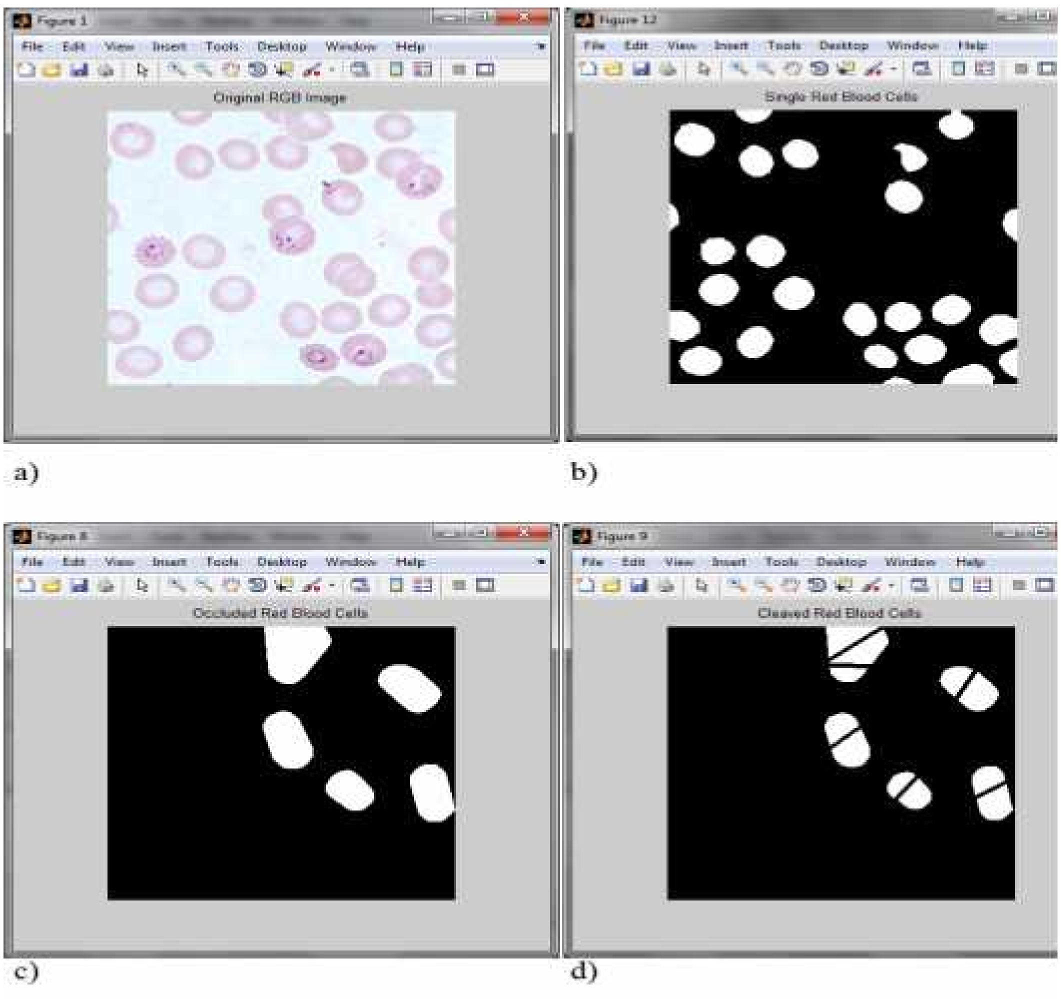

Figure 4 Matlab Results a) presents original RGB input image b) presents the single RBCs resulted as separation from occluded RBCs c) Occluded RBCs d) Cleaved RBCs after giving cuts through lines by considering boundary points

Second Input Image

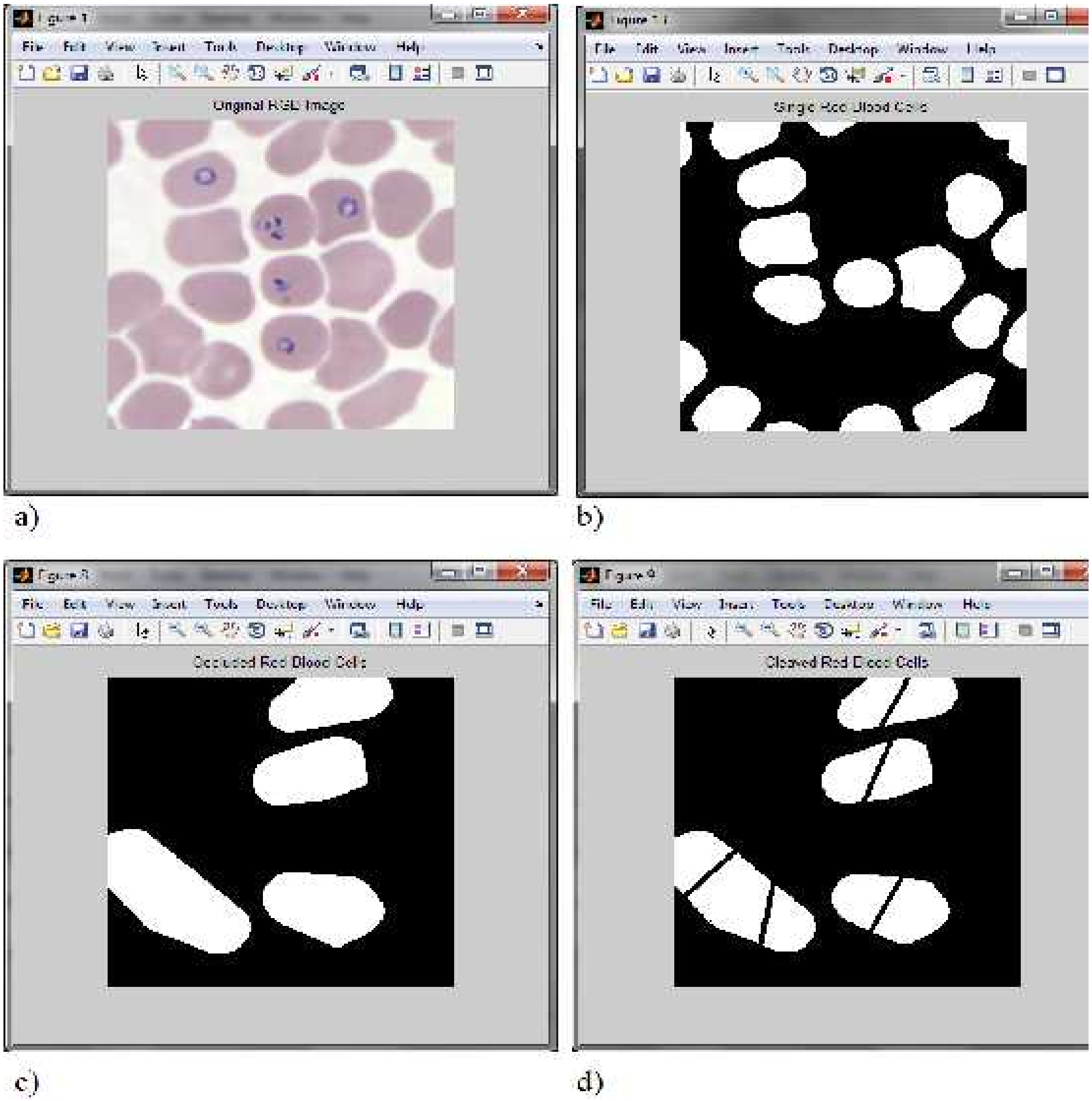

Figure 5 Matlab Results a) presents original RGB input image b) presents the single RBCs resulted as separation from occluded RBCs c) Occluded RBCs d) Cleaved RBCs after giving cuts through lines by considering boundary points

Conclusion. The occlusions splitting is an intermediate process of many computers diagnosing studies of blood and needs to be solved in an efficient and careful way because human health is involved. In this paper we produce a concept which is simple and efficient. As previously, researchers approached to the problem of occlusion of RBC successfully but computationally non-efficient ways. This study considers the mentioned point and solves the problem in a robust and real way. The proposed method is tested on a 20 images dataset obtained from DPDx [31] and we found mostly the results are good while in some high clumps i.e. more than 10 RBCs occlusion we noted a little deviation but this due to the non-smoothen boundaries. In the future goals of this study, we suggest that to smooth the boundaries through any mean as will further improve the accuracy because in high occlusion the proposed method is sensitive to noise.

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