# Rouleaux Red Blood Cells Splitting in Microscopic Thin Blood Smear Images via Local Maxima, Circles Drawing and Mapping with Original RBCs.

**Amjad Rehman[1], Naveed Abbas[2], Tanzila Saba[3], Toqeer Mahmood[4], Hoshang Kolivand[5]**

[1]College of Computer and Information Systems Al Yamamah University Riyadh 11512 Saudi Arabia
[2]Computer Science Department Islamia College University Peshawar, Pakistan
[3]College of Computer and Information Sciences Prince Sultan University Riyadh 11586 Saudi Arabia
[4]Department of Computer Science, University of Engineering and Technology Taxila-47050, Pakistan
[5]Department of Computer Science, Liverpool John Moores University, Liverpool, L3 3AF, UK

**Abstract:** Splitting the rouleaux RBCs from single RBCs and its further subdivision is a challenging area in computer-assisted diagnosis of blood. This phenomenon is applied in complete blood count, Anemia, Leukemia and Malaria tests. Several automated techniques are reported in the state of art for this task but face either under or over splitting problems. The current research presents a novel approach to split Rouleaux Red Blood Cells (chains of RBCs) precisely, which are frequently observed in the thin blood smear images. Accordingly, this research address the rouleaux splitting problem in a realistic, efficient and automated way by considering the distance transform and local maxima of the rouleaux RBCs. Rouleaux RBCs are splitted by taking their local maxima as the centres to draw circles by mid-point circle algorithm. The resulting circles are further mapped with single RBC in Rouleaux to preserve its original shape. The results of the proposed approach on standard data set are presented and analyzed statistically by achieving an average recall of 0.059, an average precision of 0.067 and F-measure 0.063 are achieved through ground truth with visual inspection.

**Keywords:** Rouleaux RBCs Splitting; Clumped RBCs; Overlapped RBCs; Preprocessing.

## 1. Introduction

The manual microscopy is gold standard but the process is highly subjective and laborious. Therefore, automated microscopy has got much attention of the researchers currently. In automated microscopy of blood, the main challenge is to split Rouleaux RBCs preciously. The Rouleaux of RBCs is subdivided into two types; clumps of RBCs and overlaps of RBCs. The word clump means “to glue”. RBCs glued with each other and formed long chains called Rouleaux. The formation of clumps or rouleaux of RBCs occurs due to iron deficiency in the blood(Kumar et al., 2018; Saba et al., 2013). The degree of severance of various diseases is highly dependent on the number of RBCs such as in Malaria the Parasitemia is the ratio of infected RBCs to all RBCs observed on the slide (Mushin et al., 2013). In automated diagnosing, the accuracy is highly dependent on the counting of RBCs (Rupesh et al., 2016; Al-Amin et al., 2015). On the other hand, overlapped RBCs numbers are not more than four RBCs combination and formation is just due to improper slide preparation. Accordingly, the cleavage in a proper, easy and efficient way is the need of the day (Kim et al., 2017; Waheed et al., 2016; Mughal et al., 2017a,b).

Further paper is organized into three main section, section 2 presents in depth background of the research in hand, section 3 presents proposed methodology, section 4 exhibits results and analysis, finally section 4 concludes the research.

## 2. Research background

Recently, researchers have come out with significant algorithms for splitting rouleaux of RBCs, however still further enhancements are needed to tackle the stated problems precisely. Kumar et al., (2018) work show the basic morphological operators for the rouleaux RBCs splitting while the simple morphological operators involve morphology dependent operators like a disc, opening and closing etc., the accuracy of RBCs segmentation will be abruptly disturbed during dense rouleaux of RBCs. Kim et al., (2017) splitting process is purely morphologically dependent because they consider the roundness feature of RBCs, is easily disturbed due to a slight pressure on the slide as well as through other diseases. Further, the study carried out by (Jyoti et al., 2017; Jamal et al., 2017; Iftikhar et al., 2017; Nodehi et al., 2014) used PCA (Principal Component Analysis) for feature reduction and SVM for classification; however, they ignored the splitting process that affected the accuracy in counting process while in the same way through pattern recognition did the segmentation of White Blood Cells (Subrajeet et al., 2016; Norouzi et al., 2014; Younus et al., 2015; Rad et al., 2013, 2016).

Berge et al., (2011) mentioned that in image processing counting RBCs is not a main concern, however the other issues which highly affect the accuracy are rouleaux RBCs splitting; therefore finding and splitting is performed through concavity points. Although, boundaries tracing and labelling are used to count the RBCs but the study didn't reveal how the single and rouleaux RBCs are splitted. While, (Buttarello and Plebani, 2008), carried out the counting but the separation of rouleaux RBCs splitting was not considered. In few reported studies, the clumps and overlaps of RBCs is not taken into account for splitting while counting the RBCs, however, a few researchers employed area based techniques (Owais Shaikh, 2013-2014) and (Nguyen, Duong, and Vu, 2011).

(Mahmood, Lim, Mazalan, and Razak, 2013), (Grietinfo.in, 2013), (Mahmood and Mansor, 2012) and (Ramesh, Salama, and Tasdizen, 2012) applied the Circular Hough Transform-based techniques to count and split RBCs, where RBCs was considered as circles that is not a proper way due to the fact that RBCs morphology is not static in nature and could be altered by other disease (Rahim et al., 2017a,b).

The earlier studies used the following categories of approaches to address the issue of rouleaux RBCs splitting. Few researchers applied morphological operations to split rouleauxs of RBCs (Buggenthin et al., 2013),(Prasad, Winter, Bhat, Acharya, and Prabhu, 2012),(Amit Kumar, 2012). However, these approaches fail, when deal with long chains. To handle these issues, concavity regions are detected to split the rouleauxed RBCs through lines cuts or circles drawing (LaTorre, Alonso-Nanclares, Muelas, Peña, and DeFelipe, 2013), (Tafavogh, Navarro, Catchpoole, and Kennedy, 2013), (Zhang, Sun, Su, and Pham, 2012), (Kumarasamy, Ong, and Tan, 2011), (Gurcan et al., 2009) and (Mughal et al., 2017; Saba 2017).

Although, the concavity based approaches produce good quality results but at the same time they are costly in terms of computation process. Studies of (Tulsani, 2013), (Ferro et al., 2013), (Hodneland, Kögel, Frei, Gerdes, and Lundervold, 2013), (Ferro, et al., 2013), (Schmitt and Reetz, 2009), (Schmitt and Hasse, 2009) and (Špringl, 2009) employed watershed techniques for the splitting task. Diverse model-based approaches for the same task are presented in (Köppen, Yoshida, and Valle, 2007), (Jiang, Ngo, and Tan, 2006) and (Abbas et al., 2016; Fern et al., 2017).

## 3. Proposed Methodology

In the current research, an improved technique for rouleaux (clumped and overlapped) RBCs splitting is proposed inspired from the watershed transform. In the proposed methodology, RGB image is taken as input, followed by segmentation of single and rouleaux RBCs to increase the efficiency. The rouleaux RBCs is further considered for splitting by applying distance transform, local maxima, circle drawings and mapping. The proposed methodology composed of three main stages: pre-processing, Splitting of Single Rouleaux RBCs and Splitting Rouleaux RBCs. Proposed research framework is presented in Figure 1.

### 3.1 Pre-processing

The image pre-processing is normally desired in all image analysis applications (Rehman and Saba, 2014; Saba et al., 2014a). Accordingly, the input RGB image is converted into binary image through benchmark thresholding methodology to minimize the computational cost. The small areas are detached from the binary image by labeling as noise (Saba et al., 2014b; Rehman et al., 2009). White holes in RBCs center emerged due to presence of haemoglobin. These holes are filled in the pre-processing stage to smooth line further processing.

To identify the RBCs existence, convex hulls of all RBCs are reevaluated. Moreover, equation (i) and (ii) are employed to compute areas and elongation of RBCs convex hulls respectively. Finally, a normalize variance among all the RBCs is drawn empirically.

$$\sum_{i=1}^{|X|} \alpha_i x_i |(\forall_i : \alpha_i \geq 0) \; and \; \sum_{i=1}^{|X|} \alpha_i = 1 \qquad \text{(i)}$$

Such that |X| = finite set of points, $x_i$ belongs to |X| while $\alpha_i$is weight set for $x_i$, total weights = 1 (normalized mean).

$$\text{Area} = \text{pixels total number} \qquad \text{(ii)}$$

pixels total number = pixels composed of convex hull of RBCs.

$$\text{Elongation} = \frac{\text{X}}{\text{Y}} \qquad \text{(iii)}$$

Such that X = x- axis; Y = y- axis

$$\sigma^2 = \frac{(\text{X}-\mu)^2}{\text{N}} \qquad \text{(iv)}$$

where, X stands for area and N is the number of terms in distribution.

**Figure 1** Proposed research framework

### 3.2 Splitting of Single and Rouleaux RBCs

RBCs are splitted in to single and rouleaux based on average of the measure of central tendency.

To identify single RBCs, area of every convex hull of RBCs is divided by median area. While negation of single RBC resulted in multi-RBCs mask; each RBC mask is forwarded to pixel IDX list of an input image to compute image of single RBCs and rouleaux RBCs. The results are presented in Figure (ii).

**Figure 2**. MATLAB Results a) Original RGB image b) Binary form of original image c) Single RBCs d) Rouleaux of RBCs

### 3.3 Splitting Rouleaux RBCs

In splitting the rouleauxed RBCs, initially, distance transform is computed to locate local maxima. Consequently, circles are drawn such that each circle is mapped with rouleaux via slight erosion. Finally, single RBC is segmented from rouleaux. This process is repeated as per number of central maxima to compute splitted single RBC image as exhibited in Figure (iii).

**Figure 3.** MATLAB Results a) Input binary image of rouleaux RBCs b) Distance Transform c) Local Maxima d) Centeriod Local Maxima e) Circles mapping f) The cleaved RBCs

## 4. Experimental results and analysis

In this section, results are presented, analyzed quantitatively through correlation of ground truth and confusion matrix. Moreover, visual inspection on fifty images obtained from is conducted (DPDx, 2002). Figure 4 presents correlation of ground truth and confusion matrix

Figure 4: Co-relation "Manual (Experts) Vs Proposed Technique" Count

### 4.1 Quantitative analysis

In this section, automatic counted RBCs results are compared with manually counted RBCs results. The RBCs are manually counted by experts. Through confusion matrix True Positive Rate (TPR), Accuracy (AC), Error Rate (ER) and True Negative Rate (TNR) as exhibited in equations (v), (vi), (vii) and (viii) respectively.

$$TPR = \frac{A}{A+B} \qquad \text{(v)}$$

$$AC = \frac{A+D}{A+B+C+D} \qquad \text{(vi)}$$

$$ER = 1 - AC \qquad \text{(vii)}$$

$$TNR = \frac{D}{C+D} \qquad \text{(viii)}$$

**Table 1 Quantitative Analysis**

| Slide No | Manual Exact single RBCs and RBCs in Rouleaux Count | Automatic RBCs Count after Rouleaux Splitting | TPR | AC | ER | TNR |
|---|---|---|---|---|---|---|
| 1 | 31 | 31 | 1 | 1 | 0 | 0 |
| 2 | 32 | 32 | 1 | 1 | 0 | 0 |
| 3 | 12 | 11 | 0.916667 | 0.956522 | 0.043478 | 0.083333 |
| 4 | 10 | 10 | 1 | 1 | 0 | 0 |
| 5 | 20 | 20 | 1 | 1 | 0 | 0 |
| 6 | 80 | 76 | 0.9375 | 0.967742 | 0.032258 | 0.0625 |
| 7 | 40 | 39 | 0.916667 | 0.956522 | 0.043478 | 0.083333 |
| 8 | 30 | 30 | 1 | 1 | 0 | 0 |
| 9 | 32 | 32 | 1 | 1 | 0 | 0 |
| 10 | 33 | 30 | 0.947368 | 0.972973 | 0.027027 | 0.052632 |
| 11 | 39 | 38 | 0.916667 | 0.956522 | 0.043478 | 0.083333 |
| 12 | 41 | 41 | 1 | 1 | 0 | 0 |
| 13 | 32 | 32 | 1 | 1 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 45 | 56 | 56 | 1 | 1 | 0 | 0 |
| 46 | 78 | 75 | 0.961538 | 0.980392 | 0.019608 | 0.038462 |
| 49 | 64 | 60 | 0.9375 | 0.967742 | 0.032258 | 0.0625 |
| 50 | 57 | 54 | 0.947368 | 0.972973 | 0.027027 | 0.052632 |

### 4.2 Visual Inspection

The results are evaluated with ground reality through visual inspection. Few results exhibited in Figure 5 attained through proposed RBCs splitting approach.

**Figure 5.** MATLAB Results; Images a), d) and g) present original input binarized images, while images b), e) and h) present rouleaux RBCs, The last column images c),f) and i) are single RBCs image cleaved with proposed methodology.

## 5. Conclusion

This paper has presented a novel approach to split Rouleaux Red Blood Cells precisely, which are frequently observed in the thin blood smear images. The proposed methodology composed of three main stages: pre-processing, Splitting of Single and Rouleaux RBCs and Splitting Rouleaux RBCs. Following preprocessing of Rouleaux Red Blood Cells images, distance transform, local maxima, circle drawings and mapping techniques are applied to achieve splitted Rouleaux Red Blood Cells. According to the results presented in Table 1, the overall average percentage True Positive Rate (TPR) achieved by the proposed technique is 96% and the True Negative Rate (TNR) is 4% while in the same way the accuracy (AC) achieved is 98% and an Error Rate (Error Rate) is 2%. Nonetheless, proposed technique achieved promising results in comparison to current state of the art; current research area has still quest to improve accuracy further.

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## Highlights

Rouleaux RBCs are splitted by taking their local maxima as the centres to draw circles employing mid-point circle algorithm. The resulting circles are further mapped with single RBC into Rouleaux at high accuracy, while preserving original shape.

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