ISSN (Print) : 0974-6846 ISSN (Online) : 0974-5645

Segmentation of Crescent Shape Blood Cells using Circular Hough Transform

A. M. Rahmani^{1*}, M. Y. I. Idris¹, R. Ramli¹ and H. Arof²

¹Department of Computer System and Technology, Faculty of Computer Science & Information Technology, University of Malaya, 50603, Kuala Lumpur, Malaysia; yamani@um.edu.my

²Department of Electrical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract

In this paper, we presented a semi-automated tool to analyze red blood cells. We utilized circular Hough transform for circular cells detection. In a light micrograph, the red blood cells may appear in a crescent shape that known as Sickle-cell. Thus, we proposed a technique to detect this shape and demonstrated its capability. Overall, our tool recorded 92% of accuracy with 70% and 72% of sensitivity and specificity respectively. Despite the promising performance, several limitations must be overcome and has been highlighted in the conclusions.

Keywords: Cell Count, Deformable Cell, Overlaps Cell, Red Blood Cell, Segmentation

1. Introduction

The analysis of Red Blood Cell (RBC) in light micrograph of biopsied tissue is commonly utilized by the pathologist for clinical diagnostics. In the past, the process has been performed manually by screening the tissue image containing thousands of RBC. This approach is prone to human errors and relies on the experience of the pathologist personnel. Therefore, automated analysis of RBC has been developed to improve the accuracy and speed up the quantification process.

Generally, analysis of tissue images includes preprocessing, segmentation, feature extraction, and classification. In this study, we focused on segmentation process as the feasibility and reliability of analytical imaging results depend on segmentation accuracy and the most important step in automated image analysis¹.

There are two major difficulties occluded an accurate automated analysis in segmenting light micrograph. First, a wide-ranging degree of overlapping cells hides partial contours and restricting the identification of individual cells. Second, the irregular or deformable contour of cells yields a dissimilarity of edge information that differs from the normal shape cells. These challenges required proper segmentation techniques and remain a tricky step.

The watershed approach and its variants²⁻⁶ are one of the common segmentation methods to handle overlapping cells. The traditional watershed identifies its markers that pointing to the approximate locations of cells by finding the regional minima of the gradient image. This follows by segmenting the image region into several influence zones of markers. The intensity differences between inside and outside of the cells will reflect the shape features of the cells. As the traditional watershed typically suffers from over-segmentation, combination with other method such as a rule-based approach⁷ was proposed to merge the over-segmented regions.

Another approach in segmenting overlapping cells is using graph-cut method. This approach constructs a graph by connecting the edge of pixels with similar

^{*}Author for correspondence

intensities. Then, a normalized minimum cut of the graph is determined and resulting in the segmented image⁸. In the situation when the overlapping cells have similar intensity levels, this approach becomes impractical⁹. Hence, an estimation of the mean radius of the cell has been proposed by Danek et al.¹⁰ to isolate the overlapping cell that only significant for spherically shaped objects.

Active contour has also been applied to segment overlapping and deformable shape cells. Initially, this method is designed to segment an irregular shape object. However, variation of active contour such as level-set based active contour¹¹ and the multiphase active contour¹² has been used to segment non-overlapping objects. In overlapping cases, using prior shape^{13,14} for every single object is computational demanding to segment a large number of RBC in light micrograph and not guaranteed that the boundary is properly identified.

Since RBC is estimated as quasi-circular shape, a segmentation method that based on circular detection can be very useful. Typically, Hough transforms is employed for circular object detection. In this study, we employed circular Hough transform for RBC detection in our developed tool. Furthermore, we add a deformable detection feature to recognize a crescent shape that occurs with Sickle cell. Then, the proposed study is tested with a light micrograph of RBC.

The remainder of this paper is organized as follows. Section 2 describes the workflows implemented for segmenting the red blood cells. In Section 3, we apply the workflows in a light micrograph and discuss the results. Finally, Section 4 concludes the paper.

2. Methodology

2.1 Pre-Processing

2.1.1 Image Acquisition

In this study, the light micrograph containing red blood cells with unidentified leukocytes used to evaluate our work is shown in Figure 1.

2.1.2 Image Intensification

The inconsistent color tone in light micrograph occurs due to uneven staining, imaging conditions and smear thickness. These degradations can be minimized with adaptive thresholding using Otsu's method¹⁵ during

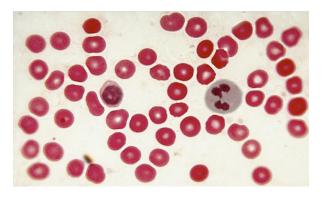


Figure 1. Light micrograph of red blood cells and leukocytes¹⁷.

pre-processing. This method will separate the pixels into two classes of background and objects from gray-level histogram resulting in binary version of input image.

2.2 Segmentation

2.2.1 Preliminary Segmentation

The circular Hough transform can be applied to recognize circular shape objects in digital images¹⁶. A circles relies on there parameters representing radius (r) and center coordinate of the circle (a,b) as follows:

$$x = a + r\cos(\theta) \tag{1}$$

$$y = a + r\sin(\theta) \tag{2}$$

To find the parameter triplets, the circular Hough transform will sweeps through 3D space to describe the circle. However, using direct implementation of circular Hough transform requires an extensive computer memory and time. If the search of circles can be reduced to 2D space with a fixed radius, the center can simply be found based on the locus point of the circle.

For instance, a set of locus points (x, y) and radius is known in x and y domain. These locus points can be transform into center of several circles in a and b domain. Through locus points of these circles, an array known as accumulator is used to detect intersection by voting for the sets of parameter that criss-crossing. Once the voting process is done, the intersection with the highest vote is defined as a center of the circle back in x and y domain. Thus, the locus of the circular object can be tracked and segment in the image.

2.2.2 Segmentation Enhancement

In circular Hough transform, it is very challenging in detecting non-circular object particularly Sickle cells that

are shaped like a crescent. Therefore, we addressed this limitation as follows.

First, the overlaps objects must be eliminated so that the crescent shape occurred due to overlapping are not mistaken as Sickle cells. The overlapping cells can be detected if the distance between two center of the circles is less than the sum of their radiuses.

Once the overlaps circles are known, the process recognition of Sickle cells continues by tracking Minima points (a thin contour) surrounding the circle of interest. If the Minima points are detected in the circle, the Hough transform will determine another circle based on the thin contour. This resulting in several overlaps circles. Then, the intersections of the new circles with the original are identified.

For example, the intersection point on the original circle that closes to the thresholded image is marked as Intersection 1 as shown in Figure 2. Next, the second intersection point (Intersection 2) is identified from the additional circle (Circle 1) that intersects with the original circle in Intersection 1. This followed by the Intersection 3 that located from the Circle 2 that intersects with Circle 1 in Intersection 2. Finally, the Intersection 4 is found from the Circle 1 that intersects with Circle 2 in Intersection 3.

In short, the intersection point is detected from the circle that crosses with another circle from the previous intersection point. The process goes until the intersection point crosses with the first intersection point.

2.3 Blob Counting

Finally, the segmented RBC in light micrograph can be counted.

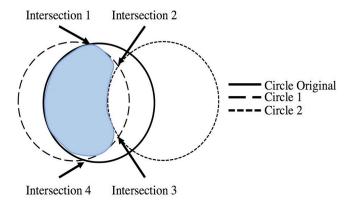


Figure 2. Four intersection points located from three circles that cross each other.

3. Results and Discussion

As described in Section 2 Methodology, the analysis of light micrograph consists of three main sections, pre-processing, segmentation and blob counting.

After the image acquisition in pre-processing, the reduction of light micrograph to a binary image using Otsu's method is shown in Figure 3. Then, the image is enhanced by removing the noises using pseudo median filter as shown in Figure 4.

This is followed by segmenting the image using circular Hough transform. In our developed tool, the user can define the range of radius that suitable for their image. This feature allowed various size or close-up image to be analyzed using our tool. The screenshot of our tool analyzing a light micrograph is shown in Figure 5.

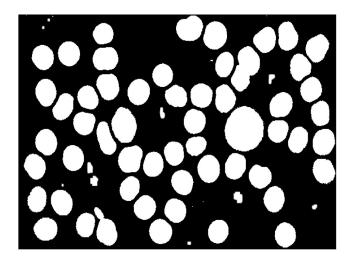


Figure 3. Binary image of RBC in biopsied tissue using Otsu's method.

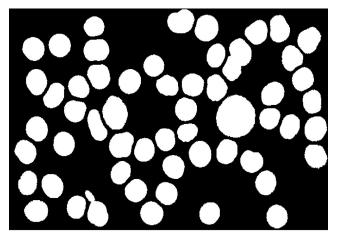


Figure 4. Noise is removed using pseudo median filter.

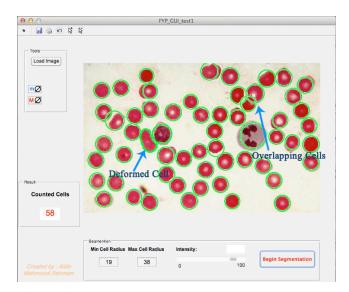


Figure 5. The light micrograph has been analyzed using circular Hough transform in our tool.

Table 1. The result of the segmentation

Cell Types	Accuracy	Sensitivity	Specificity
Normal	0.88	1	0.13
Overlapping	0.98	1	0.98
Deformable	0.91	0.17	1
Overall	0.92	0.70	0.72

In Table 1, the analysis of the light micrograph using circular Hough transform with deformable features is described. Overall, this study recorded 92% of accuracy with 70% and 72% of sensitivity and specificity respectively.

It can be observed that our analysis tool has several limitations. First, this study is designed to recognize RBC that is smaller in size compared to the leukocytes. However, if the user defined the range of radius too large, the circular Hough transform may recognize all the circular objects in the image despite its morphological characteristics. Second, if the deformable cell is overlaps with others, our tool may not be able to recognize the particular cell. Finally, our deformable detection can only recognize the cell with crescent shape. Further, if the crescent shape is not obvious, the cells may be recognized as a normal cell.

4. Conclusion and Future Works

The semi-automated tool for RBC analysis is presented. In our work, the circular Hough transform is adopted to recognize the RBC. Since, the RBC can be in crescent shape that known as Sickel cell, we proposed a simple technique for the crescent shape detection. We demonstrated our proposed study using a light micrograph and discussed the finding. Overall, our tool recorded 92% of accuracy with 70% and 72% of sensitivity and specificity respectively. Several limitation of our work also has been highlighted in the discussion section.

These limitations can be addressed in future by improving the deformable detection feature so that more deformable shape than crescent shape can be detected. Further, it will be more precise if the morphological characteristics are taken into consideration during the analysis. This will allows the tool to differentiate between RBC and leukocytes despite its size.

5. Acknowledgement

This work was financed by an UMRG grant (RP023-2012C) from University of Malaya.

6. References

- 1. Zhang YJ. A survey on evaluation methods for image segmentation. Pattern Recogn. 1996; 29(8):1335–46.
- Yan XK, Zhang N, Chen Y, Du J, Cao ZS, Zhou PK. Splitting of cell clumps in cytokinesis-blocked micronucleus images: application to improve the recognition ability of binucleated lymphocytes. Cytom Part A. 2010; 77A(8):783–9.
- Chen XW, Zhou XB, Wong STC. Automated segmentation, classification and tracking of cancer cell nuclei in timelapse microscopy. IEEE T Bio-Med Eng. 2006; 53(4):762–6.
- 4. Mao KZ, Zhao P, Tan PH. Supervised learning-based cell image segmentation for P53 immunohistochemistry. IEEE T Bio-Med Eng. 2006; 53(6):1153–63.
- 5. Koyuncu CF, Arslan S, Durmaz I, Cetin-Atalay R, Gunduz-Demir C. Smart markers for watershed-based cell segmentation. Plos One. 2012; 7(11).
- Wang YY, Sun YN, Lin CCK, Ju MS. Segmentation of nerve fibers using multi-level gradient watershed and fuzzy systems. Artif Intell Med. 2012; 54(3):189–200.
- 7. Wahlby C, Lindblad J, Vondrus M, Bengtsson E, Bjorkesten L. Algorithms for cytoplasm segmentation of fluorescence labelled cells. Anal Cell Pathol. 2002; 24(2-3):101–11.
- 8. Shi JB, Malik J. Normalized cuts and image segmentation. IEEE T Pattern Anal. 2000; 22(8):888–905.
- Felzenszwalb PF, Huttenlocher DP. Efficient graph-based image segmentation. Int J Comput Vision. 2004; 59(2): 167–81.

- 10. Danek O, Matula P, Ortiz-De-Solorzano C, Munoz-Barrutia A, Maska M, Kozubek M. Segmentation of touching cell nuclei using a two-stage graph cut model. Image Analysis, Proceedings. 2009; 5575:410-9.
- 11. Chan TF, Vese LA. Active contours without edges. Ieee T Image Process. 2001; 10(2):266-77.
- 12. Vese LA, Chan TF. A multiphase level set framework for image segmentation using the Mumford and Shah model. Int J Comput Vision. 2002; 50(3):271-93.
- 13. Chen YM, Tagare HD, Thiruvenkadam S, Huang F, Wilson D, Gopinath KS, et al. Using prior shapes in geometric active contours in a variational framework. Int J Comput Vision. 2002; 50(3):315-28.

- 14. Foulonneau A, Charbonnier P, Heitz F. Multi-reference shape priors for active contours. Int J Comput Vision. 2009; 81(1):68-81.
- 15. Otsu N. A threshold selection method from gray-level histograms. Automatica. 1975; 11(285-296):23-7.
- 16. Ioannou D, Huda W, Laine AF. Circle recognition through a 2D Hough transform and radius histogramming. Image Vision Comput. 1999; 17(1):15-26.
- 17. GRAVE SPL-E. Available from: http://www.gettyimages. com/detail/photo/light-micrograph-of-red-and-whiteblood-cell-royalty-free-image/85757384