# SEGMENTATION OF HAEMATOPOEITIC CELLS IN BONE MARROW USING CIRCLE DETECTION AND SPLITTING TECHNIQUES

*Nisha Ramesh*[†] *Mohamed E. Salama* [⋆] *Tolga Tasdizen* [†]

[†] Department of Electrical and Computer Engineering
[⋆] Department of Pathology
University of Utah, Salt Lake City, Utah, USA

## ABSTRACT

Bone marrow evaluation is indicated when peripheral blood abnormalities are not explained by clinical, physical, or laboratory findings. In this paper, we propose a novel method for segmentation of haematopoietic cells in the bone marrow from scanned slide images. Segmentation of clumped cells is a challenging problem for this application. We first use color information and morphology to eliminate red blood cells and the background. Clumped haematopoietic cells are then segmented using circle detection and a splitting algorithm based on the detected circle centers. The Hough Transform is used for circle detection and to find the number and positions of circle centers in each region. The splitting algorithm is based on detecting the maximum curvature points, and partitioning them based on information obtained from the centers of the circles in each region. The performance of the segmentation algorithm for haematopoietic cells is evaluated by comparing our proposed method with a hematologist's visual segmentation in a set of 3748 cells.

***Index Terms***— Bone marrow, haematopoietic cells, morphology, circular hough transform

## 1. INTRODUCTION

Haematopoietic cells in the bone marrow give rise to all of the blood cell types. They are grouped into erythrocyte or normoblast differentiation series, leukocyte differentiation series and megakaryocytes. A bone marrow evaluation helps to evaluate blood cell production, diagnose leukemia, bone marrow disorder and stage a variety of other types of cancer that may have spread into the marrow. Image segmentation is a difficult and challenging problem due to the complex appearance of these cells. There is fairly a wide variation of size and shape of nuclei and cytoplasm regions within given cell classes, making the segmentation problem a bigger challenge. Furthermore, cells frequently overlap each other.

The method used in this paper is different from existing approaches [1, 2, 3] : we differentiate clumped cells from single cells so that our circle detection and cell splitting algorithm is only applied to clumped cells. Furthermore, in our method, we eliminate the red blood cells (RBCs) before detecting the haematopoietic cells.

## 2. RELATED WORK

As proposed by Ongun et al.[1], haematopoietic cells can be segmented with active contour models initialized using morphological operators. This method works well only if haematopoietic cells are distinctly separate from the RBCs and have a dark cytoplasm. In our method, we eliminate the RBCs before detecting the haematopoietic cells. In [3], Park and Keller introduced a technique based on the Principle of Least Commitment for the segmentation of the leukocyte differentiation series in the bone marrow. The watershed algorithm is used to perform an "over-segmentation". The patch label memberships were relaxed in order to obtain more consistent labels for merging into cell objects. Our segmentation approach is more general since it works for all types of haematopoietic cells. Similarly, in [2], Sobrevilla et al. used an approach based on fuzzy techniques to segment the leukocyte differentiation series in bone marrow. An algorithm based on morphological watersheds was proposed by Malpica et al. and tested on the segmentation of microscopic nuclei clusters [4]. The method fails when multiple nuclei exist in a single cell. Our method uses circle detection to find the number of cells in a given region, segments cells with multiple nuclei correctly. The methods proposed by Berge et al., Kong et al., Wen et al. was studied for splitting clumped cells [5, 6, 7]. In our method, we split clumped cells based based on information obtained from the centers of the circles in each region which is novel to our knowledge.

## 3. PROPOSED METHOD

We developed an automated system for the detection and segmentation of haematopoietic cells from aspirate smears in the bone marrow. Haematopoietic cell counting is performed by pathologists only in thin sections of the aspirate smear. Similarly, as an initial step we manually select the images corresponding to the thin sections of the aspirate smear.

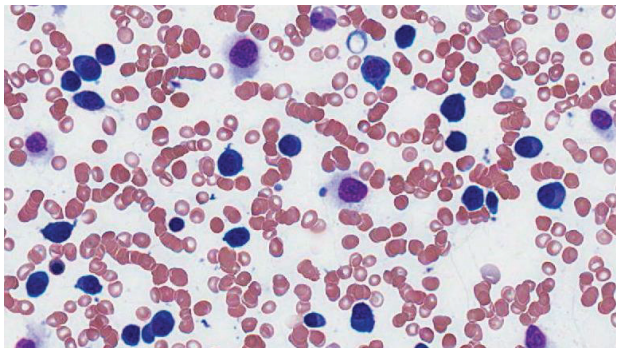


**Fig. 1**. Bone marrow sample.

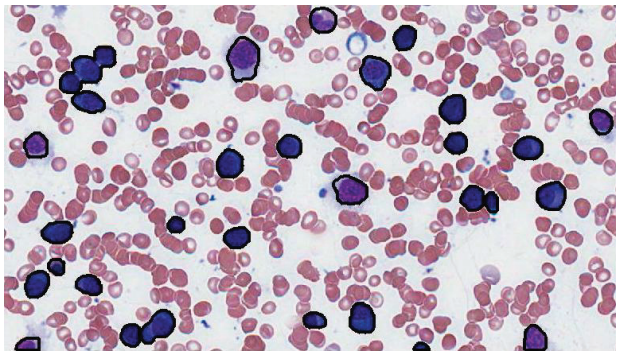


**Fig. 2**. Morphologically processed bone marrow image. The black lines represent the boundaries of every connected region.

We implemented a novel scheme for segmenting the haematopoietic cells in bone marrow. First we threshold the given image based on intensity (range 0 - 255) and eliminate small regions to obtain a binary image (threshold value = $0.7 \times 255$). This eliminates the background pixels, leaving only the RBCs, clumped platelets and the haematopoietic cells. We notice that the RBCs are mostly pink in color (without nucleus), and the haematopoietic cells have a dark stained nucleus. Therefore, we take advantage of the information present in the blue and red channels. Thresholding and smoothing the difference in the red and blue channels helps in eliminating a significant portion of the RBCs (threshold value = $0.05 \times 255$). Subtracting the RBCs and the background from the original image results in an image with mostly haematopoietic cells and clumped platelets (darkly stained). The haematopoietic cells may have possibly small parts of RBCs attached to them. The problem concerning the RBCs is addressed later. We fill in details lost due to our thresholding operation. A standard hole filling algorithm is used to fill in these details. Using a disk shaped structuring element we smooth the boundaries of the haematopoietic cells (morphological opening). Figure (2) shows the morphologically processed bone marrow sample.

Cell regions that are very close to each other are detected as a single cell by our morphological processing. We used the circular hough transform to detect the circles present in each connected region. Given the equation for a circle, $(x - a)^2 + (y - b)^2 = r^2$, the detection of circles require a 3D parameter space (a; b; r). An accumulator array is built with the following steps:

1. First we detect valid regions in the image using the color thresholding and morphological filtering procedures described above. Edge pixels are defined as pixels on the boundaries of these regions as shown in Figure (2).
2. Then, at each edge point we increment the accumulator cells corresponding to the circle in the parameter space with center in the point with the desired radius.
3. Step 2 is repeated for all admissible radii (The range of the radii is chosen by observation).

The accumulator cells then contain numbers corresponding to the number of edge points that fit the specific parameters represented by the cells. We find one or several local maxima in the accumulator by thresholding the accumulator array, which correspond to the circles in the image.

Circle detection helps in identifying the number of centers in a given region. An example of circle detection on the morphologically processed image is shown in Figure (3). Further processing of a region is based on the number of centers detected. Figure (4) represents the flowchart for the segmentation algorithm. If a single center is detected (using Hough Transform), no further processing is done, since there is only one cell in that region and the morphological segmentation is assumed to be correct. If more than one center is present then a splitting algorithm based on the information from the centers is implemented. We next discuss the splitting algorithm. The splitting algorithm can be explained in the following steps:

1. Detection of maximum curvature points on the boundary [8, 9]. Figure(5(a))

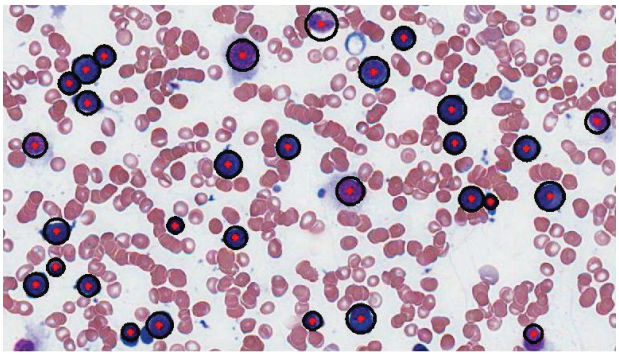


**Fig. 3**. Circle detection on the morphologically processed image. The black boundaries represents the detected circles in each region and the the red dots represent the circle centers.

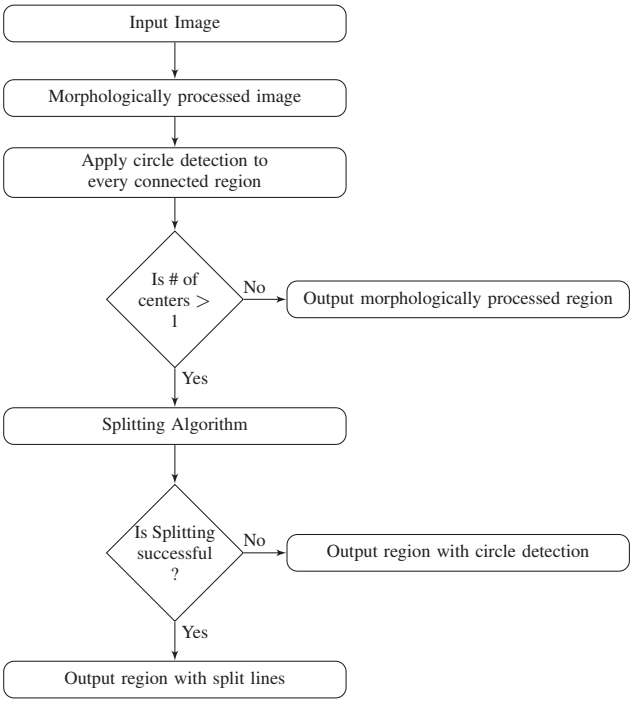
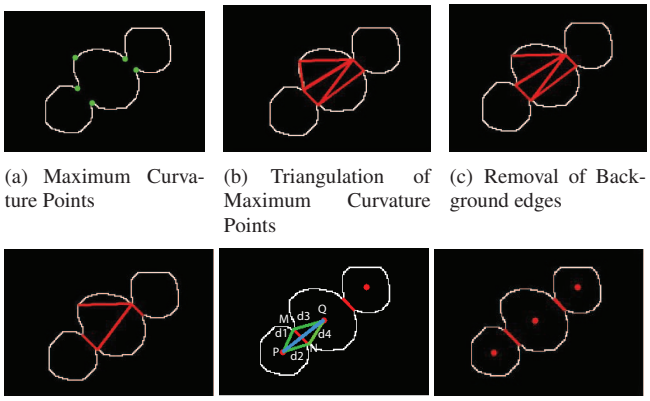



**Fig. 4**. Flowchart for Haematopoietic cell Segmentation

2. Delaunay Triangulation of Points of Maximum Curvature. This step is used to construct the set of all potential edges that might correspond to the boundaries between the different cells that are clumped together [10]. Figure(5(b))
3. Eliminate edges that pass through the background and edges that intersect the boundary. This criterion helps in retaining edges that are inside the region only [7, 5]. Figure(5(c))
4. Let $s_{ij}$ be the edge connecting two points of maximum curvature maxima $p_i$ and $p_j$. $T_i$ and $T_j$ are the unit vectors representing the tangent directions at $p_i$ and $p_j$. Eliminate edge $s_{ij}$ if $(T_i \cdot T_j) > 0$, this retains edges if the angle between tangents $T_i$ and $T_j$ is close to $\pi$ radians. These edges have the two endpoints on opposite sides of the contour, hence this is a valid edge [7, 5]. Figure(5(d))

Finally, conditions based on the information obtained from the centers of the circles in each region are used to retain the valid edges among the remaining edges. For every center P in the region being processed, the closest center Q is found. $\overline{PQ}$ denotes the line segment connecting the centers. The coordinates of the centers P and Q are denoted as $(x_p, y_p)$ and $(x_q, y_q)$, respectively. $\overline{MN}$ represents the edge under consideration. The coordinates of the endpoints M, N are $(x_m, y_m)$ and $(x_n, y_n)$, respectively. d1, d2, d3, d4 represents the lengths of $\overline{PM}$, $\overline{PN}$, $\overline{QM}$, $\overline{QN}$ respectively, Figure (5(e)).




(a) Maximum Curvature Points (b) Triangulation of Maximum Curvature Points (c) Removal of Background edges

(d) Retaining edges if the angle between tangents is close to $\pi$ radians (e) Splitting based on information from centers, dots - circle centers (f) Cell boundaries super imposed on original image

**Fig. 5**. Steps representing the splitting algorithm

The edge points M and N are considered valid when $x_m,\ x_n$ are in the range $[x_p, x_q]$ and $y_m,\ y_n$ are in the range $[y_p, y_q]$. Also, the distance between midpoint of $\overline{PQ}$ and $\overline{MN}$ should be less than a threshold $T = 3.765\ micron$. The edge is valid if $|d1 - d2| + |d3 - d4| < 6.275\ micron$, i.e the edge endpoints are equidistant from the centers, Figure(5(e)). If no valid edges are found for centers P and Q then the next closest center to P is chosen and this step is repeated. This procedure is repeated for all P.

The clumped haematopoietic cells are detected now as separate cells as shown in Figure(5(f)). The splitting technique is considered successful when the number of valid edges in a region are equal to the number of detected centers in that region minus 1. Then the split lines are used to segment the region. Otherwise, the circles from the hough transform are used to segment the region. The problem of overlapping RBCs with haematopoietic cells can now be addressed here because they are separated from the WBCs and are by themselves. We consider each of the regions detected, after splitting, threshold it at a high intensity value to retain only the nucleus. The thresholded regions are processed using morphological opening. If the number of objects in the morphologically processed region is zero then the region is eliminated.

## 4. RESULTS

This system has been tested using images obtained from the ARUP Laboratory, University of Utah. Our dataset consists of 334 images that contain 3748 expert labeled haematopoeitic cells. The images have 40X magnification. The number of circle centers detected in an image represent the number of cells detected. On comparing with visual detection, the false negatives rate was 1.62%. The false positives rate was

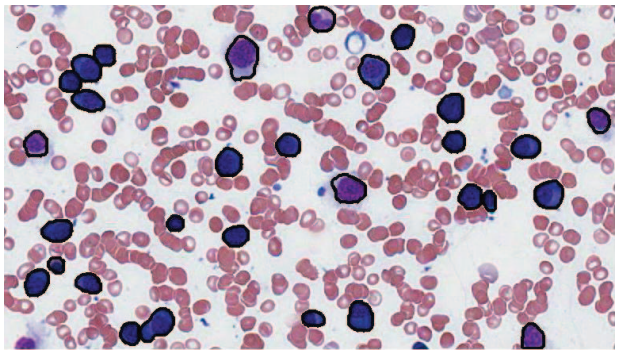


**Fig. 6**. Segmentation of haematopoeitic cells

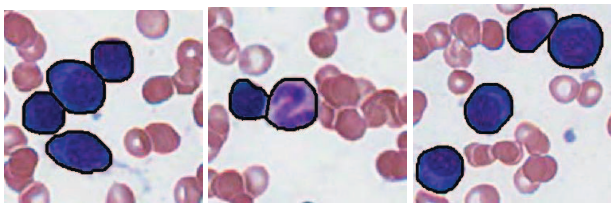


**Fig. 7**. Segmentation of haematopoeitic cells - Magnified View

1.2%. The performance of the segmentation algorithm for haematopoietic cells is evaluated by comparing the boundaries of cells from our results with a hematologist's visual segmentation, 3525 cells were accurately segmented. The accuracy rate for segmentation was 94.05 %. Figure (6) shows some segmentation results. Figure (7) gives a magnified view of the results of segmentation of haematopioetic cells. We compared the performance of our algorithm with segmentation of clumped nuclei in the bone marrow using watersheds [4]. Our method performs better and is more robust since we have tested it on a large data set in comparison with the watershed approach, Table (1). Our approach works on cells with multiple nuclei. The successful segmentation of the cytoplasm along with the nucleus segmentation aids in the automatic classification of the haematopoietic cells.

**Table 1**. Comparison of Results

| Method | No: of samples | Correct | Error |
|---|---|---|---|
| Watersheds [4] | 106 | 99(93.39%) | 7(6.6%) |
| Our method | **3748** | 3525(94.05%) | 223(5.95%) |

## 5. CONCLUSION

This paper presents a methodology to achieve an automated system for the segmentation of haematopoietic cells from scanned slide microscopic images. This segmentation technique is effective in segmenting both individual and clumped haematopoietic cells. Further studies will be focused on classification of the haematopoietic cells in the bone marrow to their subtypes.

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