Blood Cell Image Segmentation and Counting Review

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***Abstract*— The era of data and image had come as technology and computation ability improved over the last century. With the emphasis on better medical care and help of the current technology, the use of medical imaging and processing in research and diagnostics has never being this prevalent. Among which is the need to free human labor and error from blood cell classification and counting. One challenge is to separate a particular blood cell from the background (this could be saline solution, plasma, other types of cells, tissue). Preparation of the blood cell such like dilution, staining is used to aid the process of separation from the background. This project focus on the image processing part of the blood cell classification.**

Large amount of sample images taken for the blood cells is composed of mostly empty space, other biological object and often contain small percentage of the cell in interest. To solve this problem, image segmentation is used to separate the image into small attributes that contains the potential cell of interest from the background. In this project, we will first review the research efforts and methods of cell segmentation on red blood cells, white blood cells, in microcopy image and fluidics image. Then an example using image edge, color and shape as attributes to separate white blood cells in peripheral blood and bone marrow images is realized through Matlab implementation and assess the performance of the algorithm visually and quantitatively.

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***Index Terms*—Blood cell imaging, image segmentation, cell segmentation**

I. Introduction

In the late 1800s, human beings first discovered blood cell, the smallest unit of life, through observations done with microscope. With the development of hematology, blood cell images had advanced the diagnostics and understanding of human pathology such as leukemia, malaria, infection, etc. The malfunction of the human body system is often associated with blood cells' composition (WBC, RBC, Platelets, Plasma), development stages and components level change. Therefore, it is most common to get a CBC(complete blood count) in the doctors office for regular check up or first step diagnostics.

On top of the common usage of blood cell information, the potential of blood cell imaging is enormous in medical research and new studies of disease detection with the help of image feature extraction, statistics, and pattern classification.

The focus of blood image interpretation falls into two categories. The first category concentrates on replacing manual counting of RBC, WBC and platelets with computer vision and classification to generate simple counting information. The second category focuses on the study of cell morphology and pattern recognition that could provides features on specific diseases and phases detection. While the earlier uses more traditional method to acquire blood cell images such as single layer smearing and relieves the manual labor and error from lab technicians, the later one often uses more advanced image techniques such as fluorescent or reagent stain with high speed and/or resolution cameras that needs more complex image processing.

For this project, we will review both categories, provide detailed Matlab implementation of the former and thoughts on research directions for the latter.

II. Description

A. Problem Statement

The segmentation of blood cells (WBC, RBC, platelets) and counting of blood cells includes image acquisition, pre-processing to enhance image quality and minimization of noise, and counting of different blood cells in a given image. Among those processes, the most challenging one is blood cell segmentation.

B. Blood Cell Images

The images of blood cell could be obtained through variety of methods. The most common are standard microscopy, confocal microscopy, and flow cytometry microscopy [1].

Confocal microscopy generates cell fluorescence image with high resolution. It could be used to construct 3D image (Figure 1.) from layers of images. Yet the acquisition is time consuming and some areas are subjective to over bleaching or under exposure because images are taking in serial. It is widely use for research but rarely for clinical studies or diagnostics due to this reason.

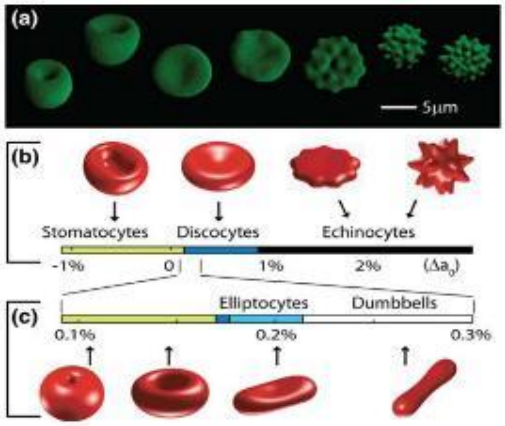

Figure 1. RBC images and theoretical shape predictions with and without a membrane cytoskeleton. (a) 3D confocal images of the canonical shapes of RBCs in different concentrations (b) Theoretical minimum energy shapes (c) Same as b, absence of a MS [3].

Flow cytometry requires cell to be in fluid suspension. The acquisition rate is rapid but in sacrifice of the image quality and fluorescence sensitivity. Several parameter including multi-directional light scatter could be detected in one event due to the suspension of the cell. And the throughput in a short time allow large population cell analysis that even the rare cell populations could be detected in statistically significant numbers.

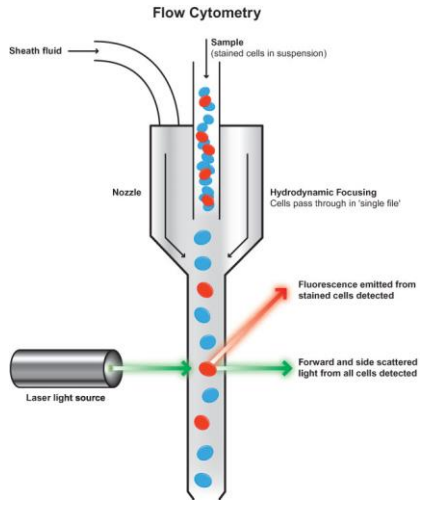

Figure 2. Flow Cytometry Illustration [10]

The standard microscopy, such as blood smear on a slide, requires manual or automated processing. Although still low in acquisition speed compared to flow cytometry, it allows different modes of cell imaging (transmitted light, scattered light, fluorescence, phase contrast) and less expensive instrumentation. Fluorescence and resolution could be comparable to confocal microscopy giving the sufficient time to integrate signal [1]. This method is still the most common practice for clinical cytological evaluations.

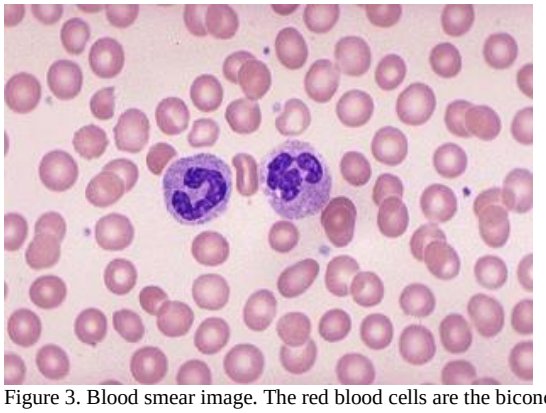

Figure 3. Blood smear image. The red blood cells are the biconcave shaped small cells. The white blood cells are the purple colored nucleated cell [3].

C. Segmentation

Image segmentation is to separate the image into sets of pixels that are meaningful for analysis. It locate the objects and boundaries (lines, curves, etc) and assign labels to pixels so that pixels with the same label form a segment. From the segments, further characteristics such as color, shape, texture and intensity could be extracted for analysis [5].

D. Segmentation Methods Review

The common segmentations methods includes morphological methods, granulometric methods, supervised methods and supplementary methods.

Morphological operations are commonly used such as image dilation, image erosion, image filling, and boundary extraction. Image dilation and image erosion correspondingly make the object fatter and thinner around the boarder. Image filling fills in the hole and merge the areas together. Boundary extracted could be added to/subtracted from the original image to sharpen the edges [4].

Granulometric method uses analysis to extract blood cell size information such as area, eccentricity, convex part area, perimeter, etc. Nucleus segmentation can be performed using level set method and watershed segmentation [6].

Supervised methods uses multilayer neural network for blood classification and counting. The network is provided with adequate training with sets of labeled cells images or characteristics [5].

Supplementary methods includes contrast enhancement preprocessing, histogram equalization and thresholding to get efficient segmentation [9].

E. Implementation

We use histogram threshold segmentation method, which image segments are separated based on thresholding of image luminance. The program used is Matlab2016a.

The dataset is taken from Ref [9]. This dataset includes 38 blood smear images that is not stained. The original image is shown below (Figure 4.).

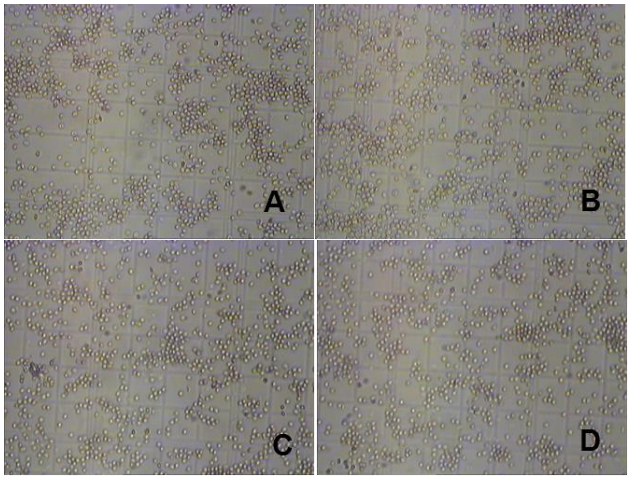

Figure 4. The 4 original images are selected randomly from the image sets. The exposure in those images is different where C and D look unevenly exposed.

The image is first transferred from RGB to green grayscale image for easy and faster data processing, because the green image has the best contrast (Figure 5).

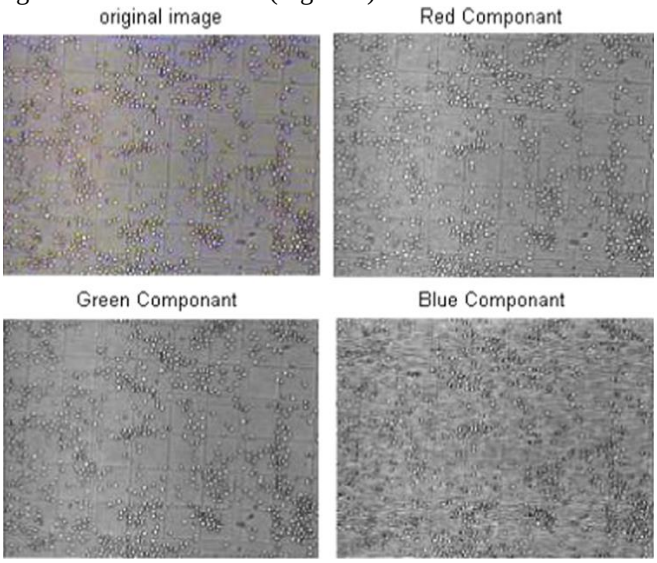

Figure 5. Color components of the original image. The Green has the best contrast [9].

We tried to use median filter of 3 by 3 neighborhood to eliminate pepper and salt noise on the green grayscale image. The filtered image is more blurred than the original image without much improvement on noise. We choose to use the unfiltered image because it has better contrast.

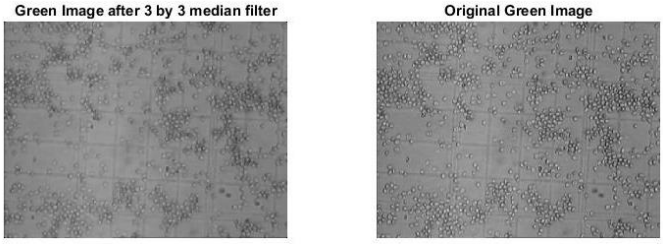

Figure 6. Green image after mediam filter and original green image.

Histogram are generated for the previous selected 4 images. As observed from the picture in Figure 7. The image quality is consistent from one to another with intensity accumulated from 100 to 200 levels, Figure 7. To enhance contrast, we could do a histogram equalization because the histogram distribution is very narrow.

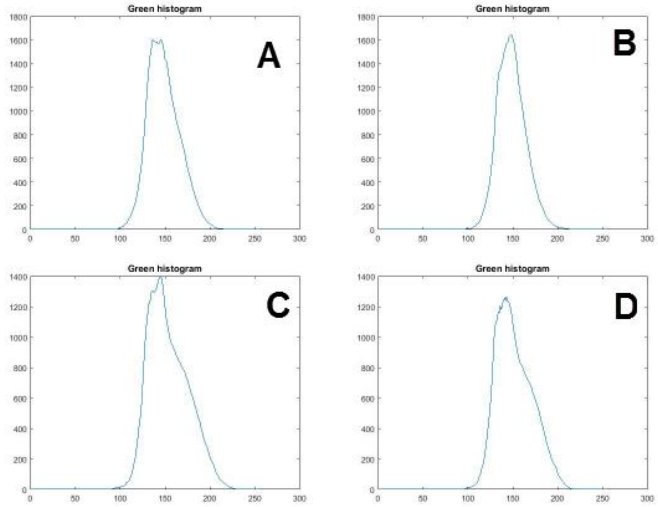

Figure 7. Green Historgram from 4 selected images.

To select the pixel threshold for segmenting RBC cells to binary image. Initial threshold is set and iterated until the segmented pixels are less than 10. The number is determined by observation and may have a variance from image to image.

Finally, mask function is used to generate the binary function and areas that is labeled as 0 pixel are counted to get RBC count.

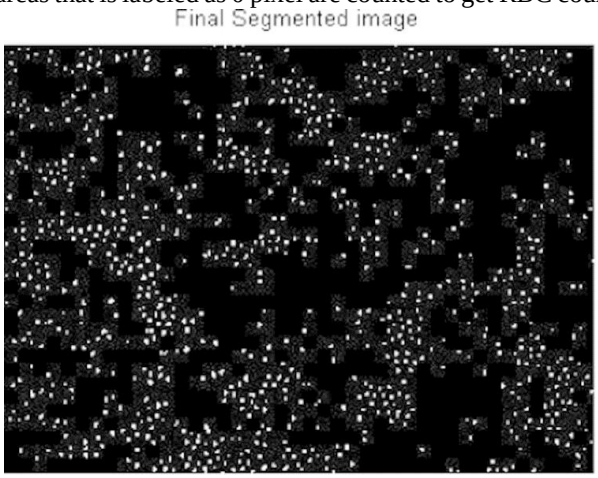

Figure 8. Final Segmented Image [9].

III. Evaluation and Discussion

A. Visual Evaluation

The images shown below is the resulting binary image compared to the original image, Figure 9.

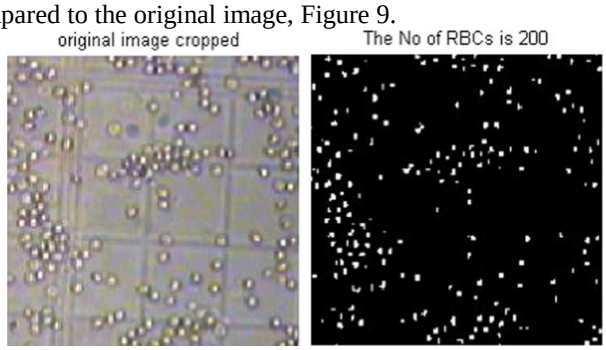

Figure 9. Original cropped Image vs. Binary cropped image [9].

We can see in the above image (Figure 9) that some of the RBC cell that has a shallow in the middle due to biconcave shape is segmented to 2 cells. This could be improved if hole filling is use to process the binary image.

However, the image below (Figure 10) has a manual count of 168, much more than the automatic count. Due to image out of focus, many RBC are dimmer and below the threshold, therefore not showing on the binary image. A focus correction function and contrast enhancement could be applied in this situation to help differentiate between RBC and background.

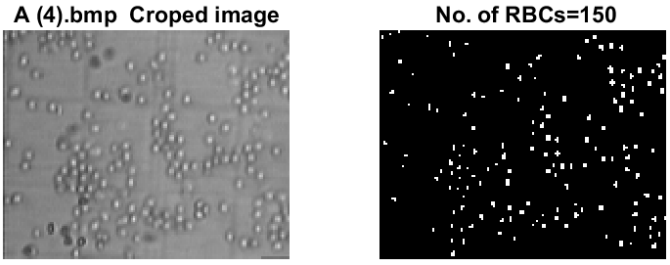

Figure 10. Original cropped Image vs. Binary cropped image, another set.

Overall the binary image reflects the location of RBC well.

B. Quality Evaluation

The cropped images are randomly selected to do manual check against the Maltab generated count, Table 1.

Manual Count	Automatic Count
174	179
168	150
168	176
157	150
182	167

Table 1. Manual Count vs. Automatic Count

More data needs to be collected to assess the success rate.

IV. Future Studies

A. Blood Cell Imaging

Blood cell images could be obtained through flow cytometry to improve the speed and accentuate the features required such as fluorescence sensitivity and light scatters. This is the future of cell imaging due to its high speed and large data.

B. Segmentation

The segmentation could be improved significantly if the image is preprocessing to enhance contrast, remove noise, focus corrected. Watershed declustering segmentation could also be applied to improve the accuracy of segmentation.

V. Summary

The segmentation method is proving to be effective in blood cell counting.

References

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