

A Self-adapting Method for RBC Count from Different Blood Smears Based On PCNN and Image Quality

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Abstract—Microscopic image processing is critical aspects to biomedical image analysis, and blood cell counts are very important role in medical diagnoses. Various dyeing methods and microscopes are used, so we need methods that can effectively count cells by adapting to such diversity. This paper presents a new method that extracts the contours of red blood cells based on the quality of a binary image that is preprocessed using PCNN. The method solves the various blood smear issues caused by the different cell dyeing methods. Moreover, it uses a self-adapting method for counting cells, using the circular Hough transform (CHT) for different amplifications. The experimental results show that the proposed method performed better in contrast variations between cells and background. The method is also much more efficient in segmentation on overlapped cells, and much more accurate in counting RBC results.

Keywords—RBC Count; a Self-adapting Method; PCNN and Image Quality.

I. INTRODUCTION

A Red Blood Cell (RBC) counts is one type of blood test aiming to provide the quantities of RBCs in a person's blood. Normally the quantities of RBCs should be between 4.7–6.1 million cells per microliter (cells/mcL) for males, 4.2–5.4 million cells/mcL for females, and 4.6–4.8 million cells/mcL for children. The RBC count is important auxiliary information for doctors to diagnose blood-related health conditions such as iron deficiency anemia (less red blood cells than normal), hemolytic anemia, polycythemia and so on. A low RBC count, indicating deficiencies in vitamin B6, B12, and folate, can signify internal bleeding, kidney disease, or malnutrition which is the patient's diet does not contain enough nutrients to meet the body's need. A large RBC count can be caused by many factors, including smoking, congenital heart disease, dehydration (e.g., from severe diarrhea), low blood oxygen levels (hypoxia), or pulmonary fibrosis (a lung condition that causes scarring of the lungs) [1].

Traditional methods for recording RBC count are manual, so they are time-consuming, subjective, and inconsistent. Recently, the development of digital cameras has led to the applications of automatic image analysis in RBC count, which

is much more faster and less subjective measurements. Many researchers have focused on obtaining accurate results from images, most of them have used traditional image processing methods. Among traditional image processing methods, the most popular methods are various segmentation and extraction techniques. An automatic method using mathematical morphology to fill RBC holes has been proposed [2], which can count overlapping cells and produce good results in most cases. The watershed algorithm [3] has been used to segment overlapping cells, but it leads to over segmentations. The fuzzy cellular neural network method [4] has been proposed for white blood cell detection. Results show that the method can detect almost all white blood cells, and the contour of each detected cell is nearly complete. The support vector machine (SVM) [5] classification has been presented to separate cells from the background, but it requires training data before the classification. The template matching algorithm [6-8] has been proposed to find and count overlapping cells, but it is inefficient and requires large quantities of samples to obtain reasonable results. A new method has been proposed [9] to segment blood cell images and count RBC based on PCNN (Pulse Coupled Neural Network) and autowave methods. Results show that the new method not only de-noise and segment blood cell image perfectly, but also well eliminate disturbed objects which will serious impact the blood cell counting step. Meanwhile, it is able to segment specific isolated cell from the background. By achieving perfect results, PCNN is very suitable for image segmentation in RBC count [11-13].

Overall, all these methods processed images of cells dyed obtained by same technique at the same microscopic scale. Additionally, these methods achieved less accurate results when applied to images of overlapping blood cells. Thus in this study, a new method has been proposed to process images obtained by different staining techniques at different microscopic scales, aiming to improve the efficiency and accuracy in counting marginal/overlapping RBCs.

II. METHOD

A. PCNN algorithm

In this study, the PCNN algorithm has been applied to convert the RGB image to binary image, and segment the RBC image. Differing from general artificial neural networks, the PCNN only has a single layer formed by a 2-D array of laterally linked pulse coupled neurons without training [14-17]. A simplified neuron model of the PCNN has been shown in Fig. 1, which has three parts: the input module, the nonlinear modulation module, and the pulse generating module. The formulas are described as follows.

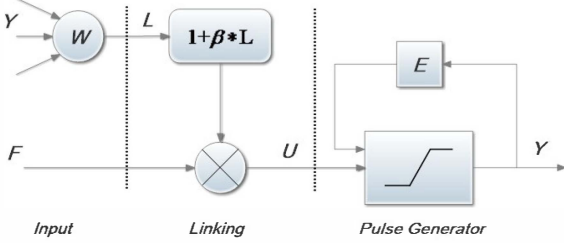


Fig. 1. PCNN Model

$$F_{i,j}[n] = e^{-\alpha_F} F_{i,j}[n-1] + V_F \sum_{k,l} M_{i,j,k,l} Y_{k,l}[n-1] + I_{i,j} \quad (1)$$

$$L_{i,j}[n] = e^{-\alpha_L} L_{i,j}[n-1] + V_L \sum_{k,l} W_{i,j,k,l} Y_{k,l}[n-1] \quad (2)$$

$$U_{i,j}[n] = F_{i,j}[n](1 + \beta L_{i,j}[n]) \quad (3)$$

$$E_{i,j}[n] = e^{-\alpha_E} E_{i,j}[n-1] + V_E Y_{i,j}[n-1] \quad (4)$$

$$Y = \begin{cases} 1 & U_{i,j}[n] > E_{i,j}[n] \\ 0 & \text{else} \end{cases} \quad (5)$$

where α_F , α_L , and α_E are the time constants; V_F , V_L , and V_E are the magnitude adjustments; β is the linking strength of the PCNN. Each neuron is denoted with indices (i, j) , and one of its neighboring neurons is denoted with indices (k, l) . Feeding F is combined with linking L as neuron's internal activity U . The neuron receives input signals via feeding synapse $M_{k,l}$, and each neuron is connected to its neighbors such that the output signal Y of a neuron modulates the activity of its neighbors via linking synapse $W_{k,l}$. When a neuron fires at first, it begins to communicate with its neighbors and encourages its nearest neighbors to fire in the way that is supported through interconnections $W_{k,l}$. When the internal activity U is greater than the dynamic threshold E , the corresponding neuron fires, otherwise the neuron remains unchanged. The internal activity U consists of the feeding input and the linking input, then its value depends not only on F but also on L , which means the state of a neuron is affected by the state of its neighborhood. So if a neuron is activated, at the next iteration those pixels which have similar intensity around it will be activated too. This is an important property of PCNN which we use to segment images.

B. Method based on image quality

A new method, MIQ (Method based on Image Quality) has been proposed based on the image quality [18,19]. MIQ first extracts contours of cells including both isolated and

overlapped cells, then gets RBC count by counting cells near the edges of the image, as well as isolated cells and overlapped cells.

The quality of image was described as formula (6).

$$m = \sum_{i=1}^M \sum_{j=1}^N S_{i,j} \quad (6)$$

where, S_{ij} is the intensity of the pixel (i, j) . Thus, we can define a centroid of an image as formula (7).

$$i_c = \frac{\sum_{i=1}^M \sum_{j=1}^N i S_{ij}}{\sum_{i=1}^M \sum_{j=1}^N S_{ij}}, \quad j_c = \frac{\sum_{i=1}^M \sum_{j=1}^N j S_{ij}}{\sum_{i=1}^M \sum_{j=1}^N S_{ij}}. \quad (7)$$

The centroid position has been used to describe the distribution of the pixel quality. If partial image has been considered, the partial centroid represents local pixel quality distribution. To find the centroid of each cell, a square matrix which has odd numbers of pixels in its edges, has been defined starting with a minimum size. Using this matrix, the entire image would be scanned to provide centroid for each local area. If the centroid of the local area locates in its center, the center point represents all the other points in this area, thus the point would be saved for further processing. If the centroid does not locate in its center, the center point can not represent the other points, thus the point would be discarded. With the same matrix size, the scanning should be repeated and the descend rate would be determined. The iteration would continue until the centroid numbers keep the same or descend rate meets threshold value.

Then, the matrix size would be increased and scan the image according to previous steps. The processing stops when the descend rate of total number becomes sufficiently steady, to avoid obtaining excessive large area comprising of many small areas. Finally, the binary image would be obtained according to suitable matrix size. Fig. 2 shows the flow chart of this algorithm.

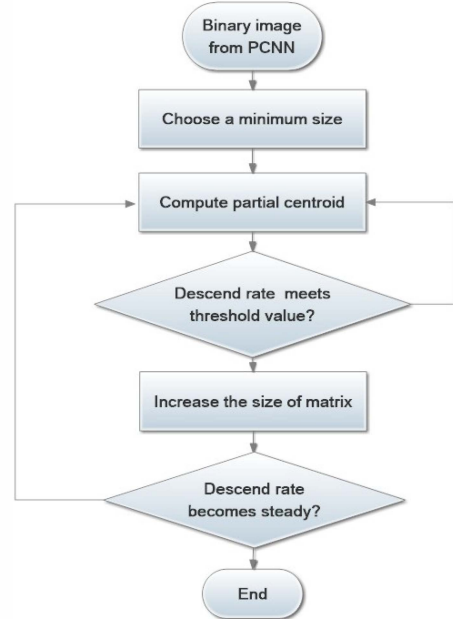


Fig. 2. Flow chart of the algorithm

As shown in Fig. 3(a), the MIQ method has been applied to the ideal cell image (isolated single RBC is an annulus). Fig.3(b) shows the average contour of a cell, which has been found by contracting the outer contour inward and extending the inner contour outward. Because the outer and inner contours in RBC image are quite near each other, so the average contour is similar to the real contour, thus the average contour would be used to describe the size of the RBC cell.

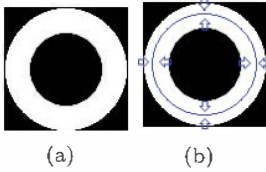


Fig. 3. (a) Ideal shape of a RBC. (b) The outer contour contracts and inner contour extends.

III. ALGORITHM AND RESULTS

As shown in Fig.4, the methods in this study process the blood cell microscopy image to automatically count the number of RBCs. First, the PCNN segmentation algorithm has been applied to convert the RGB(red, green, blue) image to binary image, and segment RBCs from the background [20]. Subsequently, the results have been de-noised by using morphology methods [2,21,22]. Finally, a new method, MIQ has been proposed based on the image quality. MIQ first extracts contours of cells including both isolated and overlapped cells, then gets RBC count by counting cells near the edges of the image, as well as isolated cells and overlapped cells.

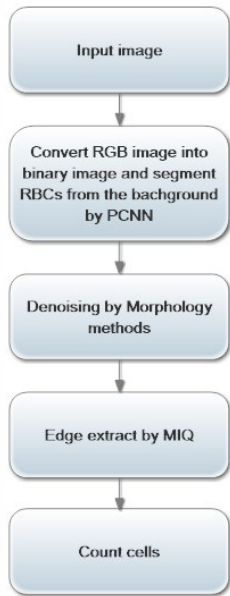


Fig. 4. The flowchart of the methods in this study

A. PCNN segmentation and de-noising

1) Image conversion and segmentation by PCNN algorithm

In a microscopic RBC image, the impacts of the overdyed

cells on counting process can not be ignored. Therefore, these cells should be eliminated. In the PCNN algorithm, the image areas of background and overdyed cells has different fire times compared with normal cells, thus the image can be easily converted to binary image, making easy extraction of the normal cells. Fig. 5 shows an example of image segmentation using the PCNN. Table I shows the PCNN model parameters in formulas (1)~(5) used in our experiments. These parameters are manually set by experiments.

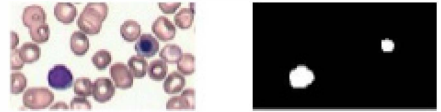


Fig. 5. Image segmentation using the PCNN

TABLE I. THE PCNN MODEL PARAMETERS

Parameters	α_F	α_E	α_L	β	V_L	V_F	V_E
Value	0.8	1.35	1	0.1	0.2	0.2	2000

2) De-noising process by morphology methods

Images are usually degraded by various noises in the signal transmission, coding and decoding processing. The results of image processing such as image segmentation, feature extraction and image recognition will depend on the noise removal results. So de-noising an image is of great importance in image processing.

To improve the segmentation result, morphology methods[2] has been applied to de-noise the converted binary image by PCNN. In this study, morphological filter has been realized by morphological operations including opening operation and subsequently closing operation. The opening operation deletes small foreground objects which do not belong to the RBC, while the closing operation eliminates all cavities. Both the opening and closing operations were performed by applying a square structuring element of size 3*3.

B. Edge extraction

The MIQ method has been applied to extracte the RBC's edge. Compared to morphology method [2], the MIQ method does not require filling in holes in cells and preserving contours, which is convenient to define the cell size in further process steps. Fig.6 shows the comparison results of both methods, the cell contour is much more complete in the processed image by the MIQ method. As shown in Fig. 7, the MIQ method performed better in segement overlapped cells with preserving their contours.

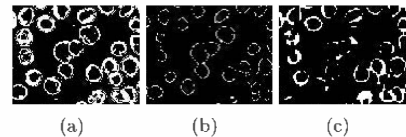


Fig. 6. Comparisons of MIQ method and morphology method (a) Original image (b) processed image by MIQ method (c) processed image by morphology method.

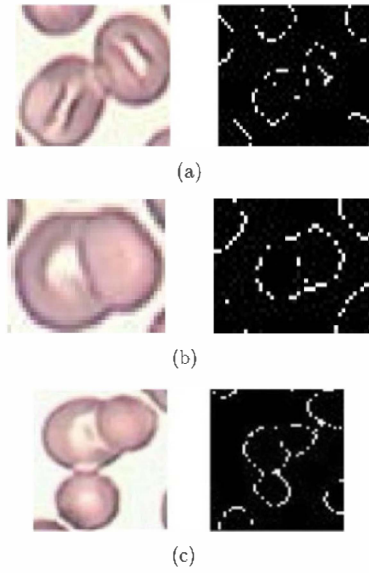


Fig. 7. the MIQ method applied to (a) mild overlapping cells, (b) severe overlapping cells, and (c) multiple overlapped cells.

C. Count cells

1) Determination of average cell size

Hough transform was proposed in 1962 to detect features of particular shapes (lines or circles) in digitalized images [23-26]. The circle Hough transform (CHT) usually has been applied to determine the radius of cells. In this study, the radius for each cell in the binary has been determined by CHT method, and the average radius of all cells has been used as the RBCs' average size. Although the average radius may be smaller than the real RBC size, it is still sufficient to accurately estimate the template.

2) Cell counting

First, a template has been construct using the RBCs' average size. As shown in Fig.8(b), a matching map would be obtained by convolving the template with the binary image. Therefore, each point in the matching map can be regarded as a cell. The template matching approach can find isolated cells, overlapping cells, and the cells only partly shown in the image.

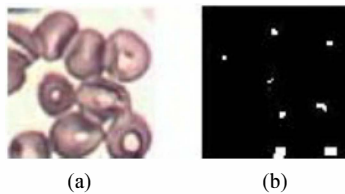


Fig. 8. Result of template matching

D. Method performance

Some typical samples have been used to compare the proposed method with the method based on PCNN and autowave[9]. As show in table II, the proposed method performs better in contrast variations between cells and background. It is also much more efficient in segmentation on overlapped cells, and much more accurate in counting RBC results.

TABLE II. TYPICAL EXAMPLE RESULTS

Experiment al image	Actual count	Result of proposed method	Accuracy of proposed method	Result of PCNN and autowave method	Accuracy of PCNN and autowave method
	22	22	100%	30	73.33%
	6	6	100%	4	66.67%
	18	20	88.89%	15	83.33%
	45	46	97.78%	1	2.22%
	45	42	93.33%	25	55.56%

Taken experiments of 45 images, the average accuracy rate of the proposed method achieves 93.18%. The proposed method performed well for both various blood smears and different microscopic scale images.

IV. CONCLUSION AND FUTURE WORK

In this study, a new method based on PCNN and image quality has been proposed, which extracts the contours of RBC from various microscopic images of disparate blood smears. The new method also extracts the contours of overlapping cells. The proposed method compared with the method based on PCNN and autowave[9] performs better in contrast variations between cells and background. Taken experiments of 45 images, the average accuracy rate of the proposed method achieves 93.18%. Compared with mathematical morphology methods, the proposed method does not require filling holes in the RBCs or prior knowledge of the cell characteristics. Compared with neural networks[27] and support vector machines (SVM), the proposed method does not require training. The proposed method is efficient and accurate.

However, the template in cell counting is based on the average size of all RBCs in the image, which may mismatch some cells in the image. Furthermore, if cell radius is much larger than the average size, the cell could be matched more than once, resulting in RBC count larger than the true value. In future work, we will improve the template matching approach and find a more efficient and accurate way to count overlapping cells and extreme large cells.

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