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POLAR ANGLE DETECTION AND IMAGE COMBINATION BASED LEUKOCYTE SEGMENTATION FOR OVERLAPPING CELL IMAGES

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Abstract. Leukocyte segmentation is one of the essential steps in an automatic leukocyte recognition system. Due to the complexity of the overlapping cell images, methods for leukocyte segmentation are still needed. In this paper, we first construct a combined image by saturation and green channels to extract the nucleus and in turn locate a cursory circular region of the leukocyte. Then the boundary of the leukocyte is represented by the polar coordinate. We determine the overlapping area by polar angle detection. Finally, another combined image is built based on the red and blue channels of the sub image covering the overlap to segment the leukocyte. The paper reports a promising segmentation for 60 microscopic cell images.

Keywords: Leukocyte segmentation, image combination, polar angle detection, overlapping cell image

1 INTRODUCTION

Hemanalysis plays a significant role in clinic diagnosis. White blood cell provides much valuable information for quantitative analysis and early diagnoses of various diseases. The process of an automatic differential blood count (DBC) system for human peripheral blood smear usually requires four steps [1]: acquisition, detection, feature extraction, and classification. The last two steps rely greatly on the second step, so the leukocyte segmentation or detection is one of the crucial steps

in the whole system. Generally, automatic analysis of microscopic images presents significant difficulties for the following reasons [2]:

1. A cell image contains not only leukocytes, but erythrocytes, platelets and various types of grunge as well.
2. Image quality is significantly affected by staining and illumination inconsistencies.
3. Most cells frequently overlap each other, and there is no clear boundary between the nucleus and cytoplasm in many cases.

These issues make leukocyte segmentation a difficult and challenging problem.

Some segmentation methods for blood cell images have been proposed. Guo et al. [2] proposed a leukocyte segmentation method based on the multispectral imaging technique. A good result could be obtained by the use of a small increment of wavelength, but it is very time-consuming, and the technique needs an expensive specific multispectral imaging microscope apparatus. Jiang et al. [3] combined scale-space filtering and watershed clustering for leukocyte segmentation. Scale-space filtering is first used to extract the nucleus from a sub image of the cell image, then watershed clustering is applied in a 3-D HSV histogram to segment the cytoplasm, and finally the morphological operations are performed to obtain the entire leukocyte. Although the feature space clustering techniques can avoid the variety and complexity in an image space, it is still difficult to determine the number of clusters in advance. Wang et al. [1] proposed a new detection algorithm (NDA) based on fuzzy cellular neural networks for white blood cell detection; but the computation of this method is comparatively high. Yan et al. [4] proposed a method for segmentation of cells from the high-throughput RNAi fluorescent images. In this scheme, nuclei are first extracted from the DNA channel by using a modified watershed algorithm. Cells are then extracted by modeling the interaction between them as well as by combining both gradient and region information in the Actin and Rac channels. An energy functional is formulated based on an interaction model for segmenting tightly clustered cells with significant intensity variance and specific phenotypes. However, erythrocyte does not contain the nucleus in human peripheral blood smear image. The method proposed in [4] can not be applied to the human peripheral blood smear image, because overlaps mostly happen between leukocyte and erythrocyte. Li [5] proposed a multiscale local adaptive threshold method to extract the nuclei with shape stability functions as criterion of selecting threshold value. This scheme only applied the gray information of the cell images neglecting the significative color information. Moreover, it did not handle the overlaps between the leukocyte and erythrocyte. Other methods [6, 7, 8] have also been proposed for leukocyte segmentation, but these methods are not good at handling the problems of the overlap in the cell imaging.

This paper focuses on the overlapping cell images to propose a novel segmentation algorithm. First, a combined image is constructed by the saturation and green channels of the cell image, through which the leukocyte nucleus can be extracted.

Second, the overlap regions are detected by identifying the polar angle change of the leukocyte contour. Finally, another combined image is built to extract the leukocyte based on the sub image derived from the overlapping images.

2 OUR APPROACH

2.1 Nucleus Extraction

Two color space models, RGB (red-green-blue) and HSI (hue-saturation-intensity) are commonly used to describe the color characteristics of images. Human peripheral blood smear contains leukocyte, erythrocyte, platelets, various types of grunge and background, and the leukocyte contains the nucleus and cytoplasm. The nucleus has a strong physical adsorption and chemical affinity because it gathers highly dense nucleoprotein and nucleic acid; so the staining density of nucleus is much higher than that of cytoplasm and erythrocyte. That is, the intensity of the nucleus is the highest in the saturation channel image. Moreover, it has been discovered that the intensity of nucleus is the lowest in green channel image. To highlight the nucleus, in this paper a combined image SG is constructed by Equation (1), where S is the saturation channel image and G is the green channel image. It is considered as a two-region image including the nucleus and other objects (cytoplasm, erythrocyte and background) as shown in Figure 1 (b). It is known that global valley points of histogram are good thresholding values for segmentation; but many local valley points appear in original histogram of the combined image shown in Figure 1 (c), so the meaningful global valley point is not easy to determine. A smoothing procedure can remove these local valley points but at the same time move the positions of global valley points. Carlotto [9] suggested filtering the histogram using a series of Gaussian filters with increasing variances, namely scale-space filtering, and drawing the valley points on each level in the same figure, namely "fingerprint figure". The fingerprint of histogram is shown in Figure 1 (d) where x-axis denotes the local valley points in histogram and y-axis the variance of Gaussian filter. It hs been found that some fingerprint lines come into being while the global valley points are detected gradually. If a tracing back is taken from large to small variance, the accurate position of global valley points can be localized and regarded as the threshold for the nucleus segmentation. Segmentation of the nucleus is shown in Figure 1 (e).

$$SG = S/G \tag{1}$$

2.2 Cursory Leukocyte Area

After the nucleus segmentation, some parameters are computed by Equation (2). In Equation (2), r is the equivalent sectional radius derived from the area formula of a circle, $s = \pi r^2$, where s is the area, that is the total number of the nucleus pixels; and l is the circumference of the nucleus, the total number of the nucleus

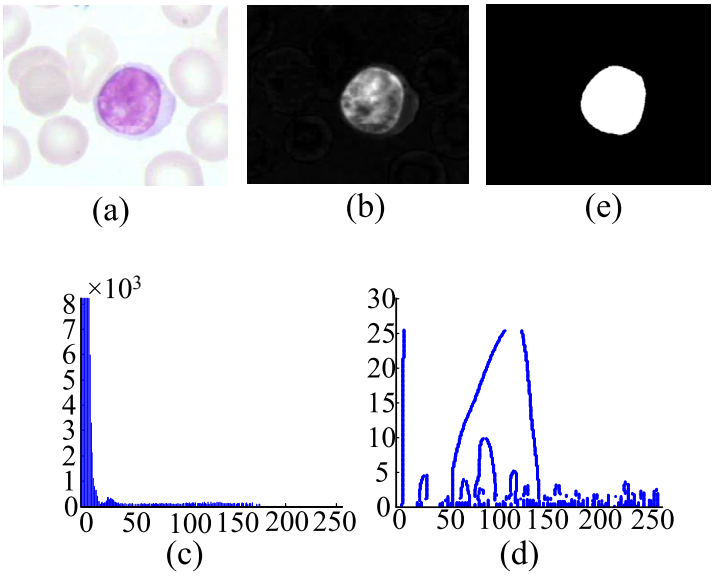

Fig. 1. Segmentation of the nucleus. (a) is an input cell image, (b) the combined image SG, (c) the histogram of (b), (d) the fingerprint of (c), and (e) the segmented nucleus.

edge pixels. The circularity is defined by $c = 4\pi s/l^2$. It is clear that $c \leq 1$. If the shape of an object is a circle, c will be 1. $f_{i,j}$ is the gray value of (i, j). x and y are the weighted centre locations.

We mark off a circular region, as shown in Figure 2 (a), where O is regarded as the centre and r_e as the radius. The circular region covers the entire nucleus and some partial erythrocytes. Note that r_e should be chosen suitably. If r_e is large, erythrocytes in the region will increase, which will lead to the difficulty of leukocyte segmentation. On the other hand, if it is small, a partial region of leukocyte will be lost, which will lead to poor precision of the leukocyte segmentation. The value of r_e is decided by Equation (3). r_e depends on the circularity c. If c is close to 1, the shape of the nucleus will be close to a circle. Such case often occurs in the two categories of leukocytes, lymphocyte and monocyte, in which the nucleus area has a big ratio and the shape is close to a circle; but for other leukocyte categories (neutrophile acidophile and basophile), the shape of the nucleus differs greatly from a circle. For the two cases, the value of r_e is different. Many experiments justify the choice of r_e defined by Equation (3).

$$r = \sqrt{\frac{s}{\pi}}, c = \frac{4\pi s}{l^2}, x = \frac{\sum_{i,j} i f_{i,j}}{\sum_{i,j} f_{i,j}}, y = \frac{\sum_{i,j} j f_{i,j}}{\sum_{i,j} f_{i,j}} \tag{2}$$

$$r_e = \begin{cases} 1.5r, & 0.85 < c < 1 \\ 2r, & c \leq 0.85 \end{cases} \tag{3}$$

In the intensity channel, the the luminance of the background region is the highest. Hence the thresholding method can be used to eliminate the background region. After that, only leukocyte and some erythrocytes are left in the image. Then, the cursory leukocyte region is located by using a priori knowledge that the leukocyte region is the biggest in the circular region, as shown in Figure 2 (b). Figure 2 (c) is its edge curve.

Fig. 2. Cursory location of the leukocyte

2.3 Polar Angle Detection

Considering that leukocyte is round in shape [10] and that a couple of concave points would come into existence in an approximate polygon of image once two cells overlap [7], we propose a polar angle detection algorithm to identify the overlap. The boundary of the leukocyte is considered as a curve by polar coordinates with the form of $\rho_i e^{j\theta_i}, i = 1, 2, \cdots, L$, regarding the centroid O as the polar point, where L is the total number of edge pixels, ρ the polar radius and θ the polar angle ranging from 0° to 360°. The first point is assigned as A. The value of θ should monotonously increase for a non-overlapped leukocyte, otherwise it isn't monotonous. While θ increases from 0° to 360°, all points in the curve are traversed. An explanation is illustrated in Figure 3. The section similar to a circle in the first column denotes the contour of the leukocyte, and the other section the contour of the erythrocyte overlapped with it. The second column denotes the polar angle curve of the cell edge pixels, where the y-axis denotes the angle θ and the x-axis the index of the edge points.

In practice, four possible overlap cases could exist according to the location between the first point A and the overlapping area contours from the end point M to N:

1. the point A does not contact with the overlapping area (Figure 3 (a1));
2. A is one point of overlapping area contours (Figure 3 (a2));
3. the line OA intersects with the end M of overlapping area contours (Figure 3 (a3));
4. OA intersects with the other end N (Figure 3 (a4)).

For the different cases, the different extreme points in the second column correspond to the two end points M and N in the first column.

It can be found from Figure 3 (a) that the angle curves in the first two cases (Figure 3 (a1) and Figure 3 (a2)) are simple but similar and prone to confusion. In view of the facts that the leukocytes are often round in shape and bigger than erythrocytes in size, the length of the overlapped edge is less than a half of the leukocyte's edge. Therefore, the angle difference of the two end points M and N is less than 180° in the first case, and larger than 180° in the second case.

However, the angle curves in Figure 3 (a3) and Figure 3 (a4) are complicated. The extreme points of the angle curve, corresponding to the two end points M and N, are not easy to determine. To overcome this, another representation of the cell edge is proposed for the two cases, as shown in Figure 3 (b). The first point is assigned as B. All points in the curve are traversed in the counterclockwise direction. The corresponding range of the angle θ is [-180°,180°]. It can be seen that the angle curve is simple and the extremum is easy to detect.

For an actual overlap, we first describe the cell edge by the polar coordinates with the first point A, then judge how many pixels are corresponding to $\theta = 0$. If there is only one pixel, the overlap belongs to either the first case or the second in Figure 3; otherwise, the cell edge is represented by the polar coordinates with the first point B, and the overlap will be performed by Figure 3 (b). Note that the peak point P_M and valley point V_N in the angle curve are corresponding to the end points M and N in the cell edge shown in Figure 3.

2.4 Overlap Elimination

In some overlap cases, it is difficult to distinguish the cytoplasm and the erythrocyte due to their similar color characters. Fortunately, it has been observed that the intensity of the erythrocyte in the red channel image is bigger than that in the blue channel image; in contrast, the intensity of the leukocyte in the red channel image is lower than that in the blue channel image. In this paper, we propose a method to eliminate the cell overlap according to the information of the red and blue channels. The sub image (Figure 4 (a)) containing the overlap region is first obtained regarding the two end points M and N as the diagonal vertexes of rectangle; then, a combined image RB is constructed by the red and blue channels of the sub image (see Equation (4)), where R and B are the red and blue values in RGB image. The R/B processing can highlight the difference of erythrocyte and leukocyte in order to separate them; finally, the fingerprint smoothing and thresholding method

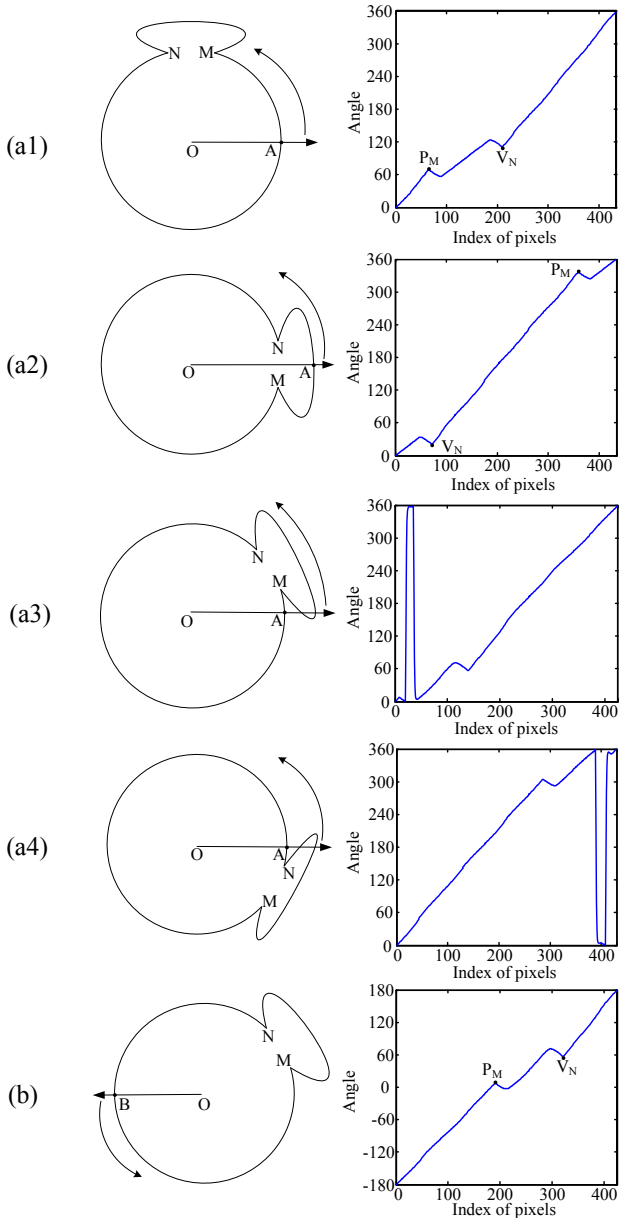

Fig. 3. Schema of the overlap cases and polar angle curves

are used to separate the overlapped cells. Figure 4 (b) shows the combined image RB, its histogram and the overlap elimination. The segmentation of leukocyte by our method is illustrated in Figure 4 (c) and by manual segmentation in Figure 4 (d).

$$RB = R/B \tag{4}$$

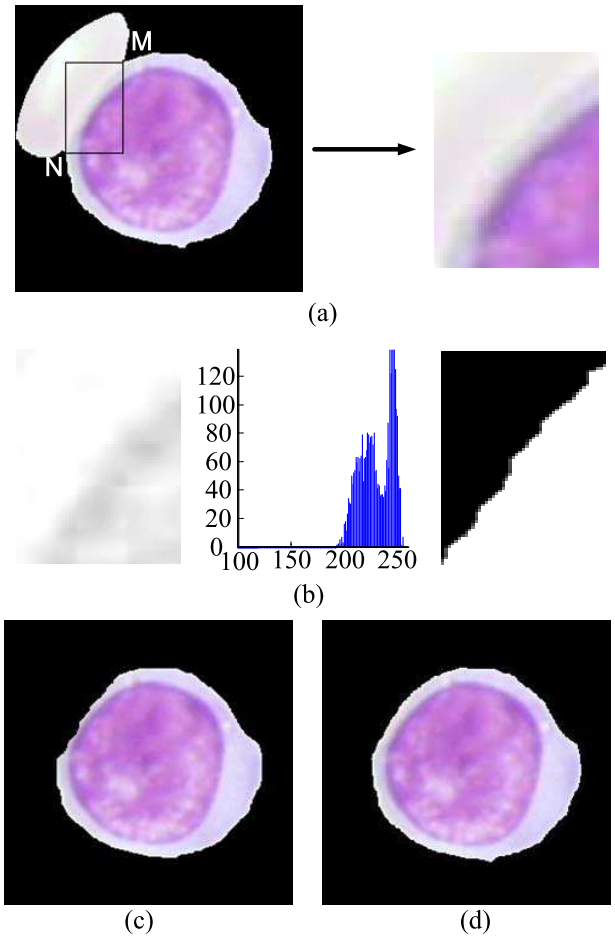

Fig. 4. Overlap elimination and leukocyte segmentation

3 RESULTS

We have tested three groups of cell images acquired by different conditions and provided by ChongQing TianHai Medical Equipment Co., Ltd. They are collected

in 24-bit RGB color, and the magnification is 10×100. The first group contains 10 images, with the size of 768×576, the second group 14 images with the size of 800×600, and the third group 36 images with the size of 1024×768. In experiments, each test image containing leukocyte, with the size of 300×300, is cropped from each of 60 original cell image.

Figure 5 gives some examples for segmentation. The top row shows overlapped cell images, the middle the manual segmentation and the bottom the segmentation by our method. Visually, our result is approximately the same as the manual one. To quantify the performances of the novel method, three parameters called P_1, P_2 and P_3 are defined. P_1 denotes the ratio of the number of the detected leukocytes (N_D) to the existing number of leukocytes (N_E) in all the cell images. $P_1 = 1$ demonstrates that all leukocytes in cell images can be detected. P_2 denotes the ratio of the number of the detected leukocytes to the total of the detected objects (N_O). $P_2 = 1$ shows no false detection. Obviously, P_1 and P_2 directly influence the detection accuracy of the whole system. P_3 denotes the ratio of the difference pixels between in the detected leukocytes and the actual leukocytes to the actual leukocyte pixels. It describes the completeness degree of the detected leukocytes, especially the edge areas. P_3 has a close relation with the classification accuracy of the whole system because the features extracted from the detected leukocytes strongly affect the classifier performance. The closer P_3 is to 0, the better the method is.

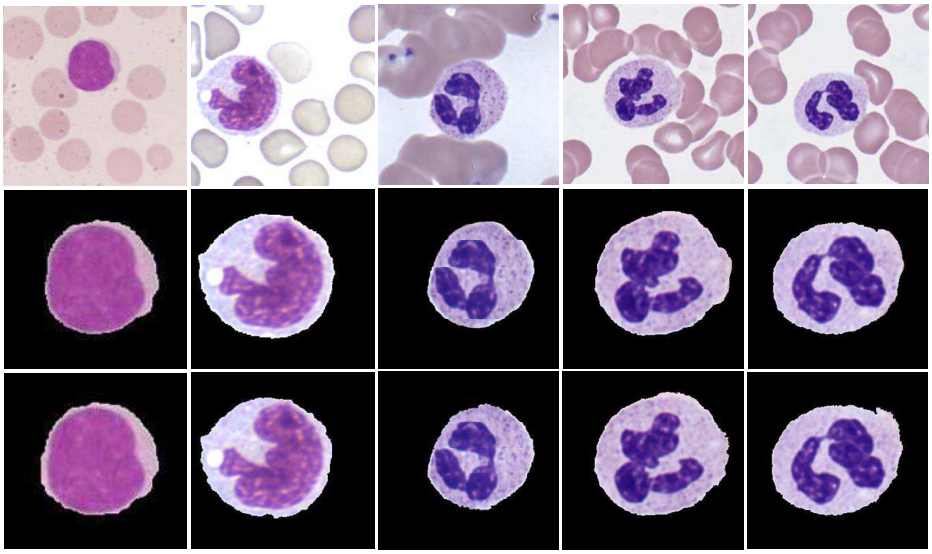

Fig. 5. Some samples of leukocyte segmentation

The performance of our proposals is evaluated in Table 1. In experiments, the actual leukocytes in the cell images are recognized and located by a professional doctor. The values of N_D, N_E and N_O are 101, 102 and 104, respectively. P_1 is

	N_E	N_D	N_O	P_1	P_2	P_3
NDA method [1]	–	–	–	98 %	99 %	6.1 %
Our method	102	101	104	99 %	97 %	2.3 %

Table 1. Performance evaluated by parameters

up to 99 %, a little better than 98 % in NDA method [1], that is, we can extract almost all the leukocytes. P_2 is 97 %, slightly less than 99 % in NDA method. The leukocyte detection starts from the nucleus detection in our method; but there is a little grunge holding the same staining property as the nucleus, and these objects are easily judged to the wrong nucleus, which can affect the value of P_2. In our method, P_3 is up to 2.3 % and outperforms 6.1 % in NDA method.

4 CONCLUSION

This paper proposes an automatic leukocyte segmentation algorithm aiming at the overlapping cell images. First, a combined image based on the saturation and green channels is constructed to extract the nucleus. Second, a polar angle detection method is proposed to identify the overlap region. To eliminate the overlap, another combined image is built based on the red and blue channels of the sub image. Eventually, the whole leukocyte was extracted perfectly. The proposed method makes the best of the information of the channels in the RGB and HSI color space models. The data such as histogram and polar angle function are all one-dimension sequences, so the procedures are easy to perform.

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REFERENCES

[1] Wang, S. T.—Wang, M.: A New Detection Algorithm Based on Fuzzy Cellular Neural Networks for White Blood Cell Detection. IEEE Trans. on Information Technology in Biomedicine, Vol. 10, 2006, No. 1, pp. 5–10.

[2] Guo, N. N.—Zeng, L. B.—Wu, Q. S.: A Method Based on Multispectral Imaging Technique for White Blood Cell Segmentation. Computers in Biology and Medicine, Vol. 37, 2006, pp. 70–76.

[3] Jiang, K.—liao, Q. M.—Xiong, Y.: A Novel White Blood Cell Segmentation Scheme Based on Feature Space Clustering. Soft Computing, 2006, No. 10, pp. 12–19.

[4] Yan, P.—Zhou, X.—Shah, M.—Wong, S. T. C.: Automatic Segmentation of High Throughput RNAi Fluorescent Cellular Images. IEEE Trans. Information Technology in Biomedicine, Vol. 12, 2008, No. 1, pp. 109–117.

[5] Li, Z.—Najarian, K.: Biomedical Image Segmentation Based on Shape Stability. IEEE International Conference on Image Processing, Vol. 6, 2007, pp. 281–284.

[6] Mukherjee, D. P.—Ray, N.—Acton, S. T.: Level Set Analysis for Leukocyte Detection and Tracking. IEEE Trans. Image Processing, Vol. 13, 2004, No. 4, pp. 562–572.

[7] Liao, Q. M.—Deng, Y. Y.: An Accurate Segmentation Method for White Blood Cell Images. Proceedings of IEEE International Symposium on Biomedical Imaging, 2002, pp. 245–248.

[8] Fang, Y.—Zheng, C. X.—Pan, C.—Liu, L.: White Blood Cell Image Segmentation Using On-line Trained Neural Network. Proceeding of the 2005 IEEE Engineering in Medicine and Biology 27th Annual Conference, Shanghai (China), 2005, pp. 6476–6479.

[9] Carlotto, M. J: Histogram Analysis Using a Scale-Space Approach. IEEE Trans. PAMI, Vol. 9, 1987, No. 1, pp. 121–129

[10] Wang, W. X.: Binary Image Segmentation of Aggregates Based on Polygonal Approximation and Classification of Concavities. Pattern Recognition, Vol. 31, 1998, No. 10, pp. 1503–1524.

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