Microscopic Cell Image Segmentation and Counting Algorithm Based on Image Definition

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***Abstract* — In this paper the problem of microscopic cell image segmentation and counting is studied, and a novel segmentation and counting algorithm based on image definition is proposed. First, the microscopic cell image is preprocessed. Second, Discrete Cosine Transform (DCT) is applied, and the high frequency signal part is achieved for differential calculation with the original image, hence to get the clear image part and the blurred image part. The target area is extracted using region growing, since the local cell image has a high clustering character. Finally, the segmentation and counting is carried out. The experimental results show that the proposed method has an accuracy above 90%, which is better than the Watershed method. Therefore, the image definition based method can successfully analyze and counting the microscopic cell image and bring a promising result.**

Keywords - Image definition, Discrete Cosine, Microscopic image, Cell counting, Image segmentation

I. INTORDUCTOIN

In biomedical engineering, the study on the relation of cell structure and function is a very important topic [1], the quantity analysis on microscopic image is one of the foundations of such study. Compared with human annotation, the microscopic image processing on biological image has a clear advantage in segmentation and counting, since it is fast and automatic. However, due to the low gray scale image and uneven distributed intensity, as well as the complicated cell structure, the automatic segmentation and counting is a challenging task [2-3].

Su et al., proposed PCNN automatic wave feature based blood cell image segmentation and counting, the number of the blood cells is counted accurately and special type of cells are segmented independently [1]. However, their algorithm is not robust when two neighboring blood cells are overlapping, and not applicable to close and special shape plant cells. Wu et al., proposed automatic counting method based on active contour model, the overlapping cells are not segmented directly and the accuracy is higher than 90% [4]. Ren et al., proposed an improved Ostu method on the image segmentation of Rat sperm, both the fast segmentation and counting are implemented [5]. However, their method is not robust to the adhesion of sperm.

Due to the complicated cell structure [6], different methods are suitable for different cell images. The background image is generally out of focus in microscopic image collection since the camera is focused on the cell. In this paper, we study the image definition of the microscopic image, and proposed a novel segmentation and counting algorithm based on image definition. Image definition refers to the clarity of the detailed texture and its edge. We use Discrete Cosine Transform (DCT) [7-9] to achieve the low frequency part of the image contour, and the high frequency part of the image details. The detailed image can be achieved by the differential calculation between the low frequency part of DCT signal and the original image. The target area is extracted using region growing, since the local cell image has a high clustering character. The analysis and counting of the microscopic cell image based on this method are accurate and effective according to the experimental results.

II. METHODOLOGY

DCT is an orthogonal transform method proposed by N. Ahmed and Rao in 1974 [10]. After the DCT transform, the signal frequency character is more significant, and the energy of the signal is more concentrated. The high frequency part of the signal is related to the details in image, and the low frequency part of the signal is related to the image contour. The definition can be achieved by getting the high frequency of DCT signal [11], and the microscopic cell image segmentation and counting[12] can be implemented according to the following steps:

1) Convert image to gray scale and denoise using median value filtering method [13], as shown in Figure.1(a);

2) Apply DCT Transform on the denoised image, as shown in Figure.1 (b);

3) Use half of the low frequency signal value as the threshold, cut the high frequency signal, and perform Inverse DCT transform to go back to the gray scale space, as shown in Figure.1(c);

4) Calculate the difference between the inversed DCT image and the original image, convert to gray scale, as shown in Figure.1(d);

5) Use the adaptive threshold method [14]to convert the gray scale differential image in Step 4) into binary image, and use 3×3 template to perform close and open operation for denoising. The discrete noise point is removed and the clear area is achieved, as shown in Figure.1 (e);

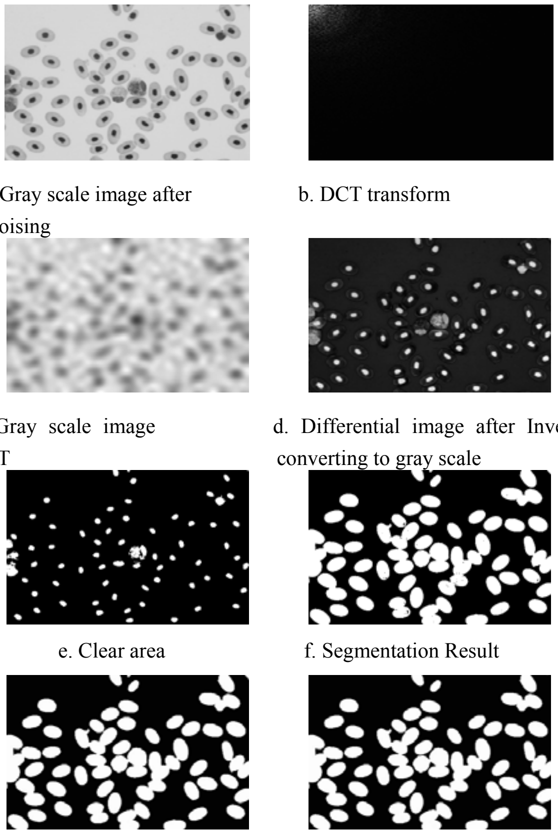
6) Based on the high clustering property of local target image, use the clear area as the initial growing area, and perform region growing method. Mark the similar gray level point on the original gray image, if the neighborhood satisfy Eq. (1), the marked pint is the front view point, continue grow; else go to the next unmarked growing point, until the marking process is finished, and the target is found, as shown in Figure.1 (f).

$$|y1(i, j) - y1(i', j')| < threshold \tag{1}$$

Where point (i, j) is the current growing point, (i', j') is the eight connected neighboring areas of (i, j). Generally, the gray scale change within the cell is very small, the edge between the cell and the background is obvious. When the threshold is too large, the over growth may happen. Therefore, *threshold* is usually set to a small value. In this paper *threshold* is set to 6.

7) There may be many holes in the cell area, and isolated noise points, therefore we perform the hole filling algorithm [15], as shown in Figure. 1 (g);

*8)*Mark the connected area, record the rectangular coordinates that fitted on the connected area and the area proportion. Cluster the areas into three classes, the smallest one is taken as noise to remove, as shown in Figure.1 (h);



a. Gray scale image after denoising

b. DCT transform

c. Gray scale image DCT

d. Differential image after Inverse converting to gray scale

e. Clear area

f. Segmentation Result

g. Denoising and hole filling

h. Remove significant noise

Figure 1. Results of each segmentation step

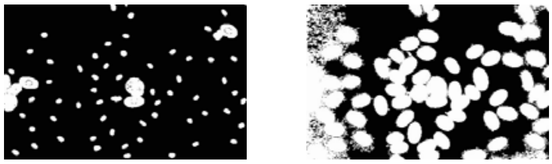
9) Take the smaller one in the rest two classes in Step 8), rank the areas and take the median value as the single cell area. Estimate the cell numbers in other connected area and get the final cell number.

III. EXPERIMENTAL RESULT

The proposed algorithm is implemented using OpenCV 2.4.8 and Visual Studio2010 on PC platform.

A. Segmentation Results Analysis

In Eq.(1), the selection of threshold value has a large influence on the segmentation result. When the threshold is too small, the front view may be missed. When the threshold is too big, the over growth may happen, and mark the wrong background as cells, as shown in Fig.2.



a. under growth

b. over growth

Figure 2. Segmentation results of over growth and under growth

Use Photoshop to do the manual annotation on the experimental image. The human segmentation result is achieved. Use p=tp/(tp+fn) to calculate the accuracy of the segmentation. Where tp is the corrected segmented cell points, fn is the mis-marked background points, the relation between the threshold and the accuracy is shown in Fig.3.

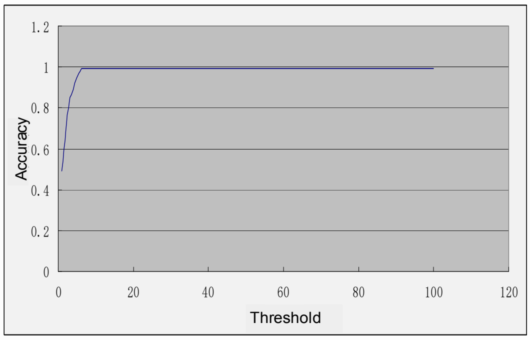
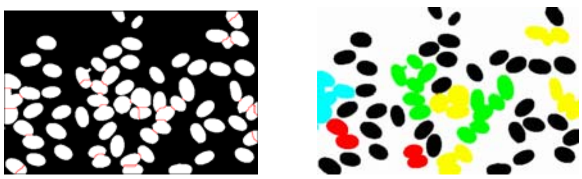


Figure3. Relation between threshold and accuracy

We can see in Fig.3 that after the threshold of the growing area is bigger than 6, the accuracy is constant. However, when the threshold increase, the over growth may happen, and the accuracy may decrease. Therefore, we take 6 as the growth threshold.

B. Counting Result Analysis



a.Watershed segmentation method

b. The proposed segmentation method

Figure 4. Comparison of counting result

Use Watershed method [16-17] as a comparison to the proposed method. Extract the connected area of the cell after applying Watershed method, separate the adherence cells using segment line and count the number of cells.

The Watershed method has a drawback in over segmentation and under segmentation, the accuracy is not high, as shown in Fig.4 (a), the red line denotes the segment line. In our method, we don't segment the adherence cells directly, we count the cell numbers based on the area of connected regions, as shown in Fig.4 (b). Different color denotes the different counting using our method.

We further carry out counting experiments on 4 different cell density, and compared the Watershed method and the proposed method in this paper. The counting results are shown in Fig.5.

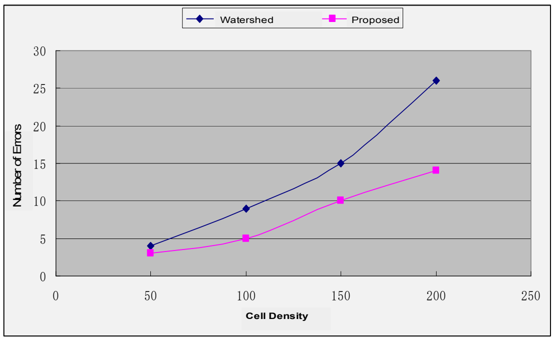


Figure 5. Comparison of error curve

TABLE 1 COMPARISON OF COUNTING ACCURACY

Density /μL \ Method	50	100	150	200	Averaged Accuracy	Averaged time (ms)
Watershed method	92.0%	91.0%	90.0%	87.0%	89.2%	114.38
proposed method	94.0%	95.0%	93.3%	93.0%	93.6%	87.47

We can see from Fig.5 that when the cell density increases the accuracy drops. When the density increases, more cells are overlapping with each other, and the segmentation is more difficult. Using the proposed method in this paper, the accuracy is higher than the Watershed method, since we don't segment the overlapped cell directly, the influence caused by cell density change is lower. The counting accuracy is higher (above 90%) as shown in Tab.1.

The experiment platform is DELL Precision M6600, CPU is Intel® Core™ i7-2960XM @ 2.70GHz (8CPUs), and Windows XP is installed. The resolution of the testing image is 500*316 pixel. Fast DCT is adopted, and the overlapping cells are not segmented directly, therefore the speech is fast. The averaged time for segmentation and counting on one image is 87.47ms. In the Watershed method, the distance transform, and converting from binary image to range image is needed, therefore the speed is slow when there are many cells. Apply Watershed method on one image, the averaged time is 114.38ms, as shown in Tab. 1.

IV. DISCUSSION

Due to the complicated structure of cells, the current segmentation algorithms are limited to the overlapping of cells, fast and accurate segmentation is a challenge. The Watershed method is influence by the noise and may cause the over segmentation and the under segmentation. In this paper, we propose to use the global image definition to segment the cell and the background, and use the local gray scale region growth to extract the target cell. The proposed image definition based microscopic cell image segmentation makes use the local color consistence, as well as the camera focus location on cell. The accuracy is improved using the global definition based cell and background classification, as well as the local clustering property and gray scale region growth. In the counting process, the area of the connected regions are analyzed, the direct segmentation of the adherence cells is avoid, and the computing speech is also improved. We may conclude that:

1) In this paper, a novel cell image segmentation method based on image definition is proposed, which is aimed at cell images with low gray scale, uneven intensity distribution, and complicated structure. It changes the traditional global gray scale segmentation in microscopic cell image, and adopt the image definition based method to search the clear part of the image. The region growth method is then used to segment the complete target cell.

2) In the traditional Watershed method, the overlapping cell segmentation is influenced by over segmentation and under segmentation, the counting error is high. As the cell density increases, the error also increases. In this paper we use the cell area clustering method to analyze the overlapping cells, and the automatic counting accuracy is above 90%. In this method, the cell segmentation is not performed directly, it is based on the statistical information of cell areas. This method decreases the error caused by overlapping cells and it is less likely to be influenced by cell density. Experimental results show that under different cell densities, the proposed method outperform the Watershed method.

3) The limitation of this cell image segmentation algorithm: the image definition based algorithm is only applicable to the well focused image. When the image is out of focus, the segmentation and counting is not reliable. In the future, we may use image enhancement method to improve the algorithm, the high pass filtering method may be used to enhance the image definition.

V. CONCLUSIONS

In this paper, a novel segmentation and counting algorithm based on image definition is proposed. the microscopic cell image is preprocessed and Discrete Cosine Transform (DCT) is applied, The target area is extracted using region growing, since the local cell image has a high clustering character. the segmentation and counting is carried out in the end. The experimental results show that the proposed method has an accuracy above 90%, which is better than the Watershed method. Therefore, the image definition based method can successfully analyze and counting the microscopic cell image and bring a promising result.

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