Cell Detection and Counting Method Based on Connected Domain of Binary Image

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Abstract. Cell counting plays an important role in biomedical research. There are always some phenomena such as indistinct intervals and target adhesion in cell images, which leads to poor segmentation effect and therefore inaccurate counting. In view of this situation, based on image binarization technology, this paper proposed a rapid cell count method combining mathematical morphology and connected domain labeling in which the cell images can be grayed, USM sharpened, binarized, morphologically processed, and connected domain labeled, and ultimately the number of cells could be calculated. The experimental results show that this method can effectively complete the segmentation of sparse cell images and intensive cell images, and the counting error is less than 5%.

Keywords: Binarization · Morphology · Connected domain · Cell counting

1 Introduction

Cell image counting is an important research direction in medical image processing. In recent years, with the development of image processing technology and machine learning methods, a large number of new theories and methods have emerged in the detection and counting of cells.

Literature [1, 2] proposed a method for automatic cell counting using Hough transform. Adherent cells were separated by morphological treatment, and cell count was realized by using circulating Hough transform. Literature [3] proposed a watershed segmentation method based on basin expansion, which can effectively segment adhesion objects and non-adhesion objects. Literature [4], the image noise is removed by the reconstruction operation of grayscale morphology, and the contrast of gradient image is enhanced by combining the top-hat transformation and the bottom-hat transformation. The cell image is segmented by the watershed algorithm, with significant segmentation effect. Literature [5], a new method of cell image segmentation based on wavelet transform and morphological watershed is proposed to solve the problems of over-segmentation when watershed segmentation algorithm is used. Literature [6] proposed a new blood cell image segmentation and counting algorithm by studying the PCNN and Autowave characteristics, which effectively eliminates the influence of small interference on cell image segmentation. Literature [7] proposed an image segmentation method that combines edge detection and mathematical

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morphology, which effectively makes up for the shortcomings of a single segmentation algorithm, such as the lack of fine edge segmentation and the large number of holes in cells. In literature [8], K-means clustering was used to segment cell images under HIS color model, and the results showed that this method could remove background noise and retain target images well, thus achieving a good segmentation effect. Literature [9] discussed and evaluated four counting methods of K-means clustering, watershed transformation, histogram equalization and detection based on shape features. The experimental results showed the advantages and disadvantages of each method. Literature [10] proposed an automatic counting system of red blood cells based on fuzzy C-means clustering. Image filtering was carried out by fuzzy clustering method to separate red blood cells from background cells. After morphological processing, the counting accuracy was relatively high.

The above methods usually require a large amount of computation and take a long time to count. In this paper, a fast, intuitive and simple cell counting method is realized by programming graphical user interface (GUI) based on Matlab language, as shown in Fig. 1. This method can effectively conduct cell detection and more accurate cell count. The flow chart is shown in Fig. 2.

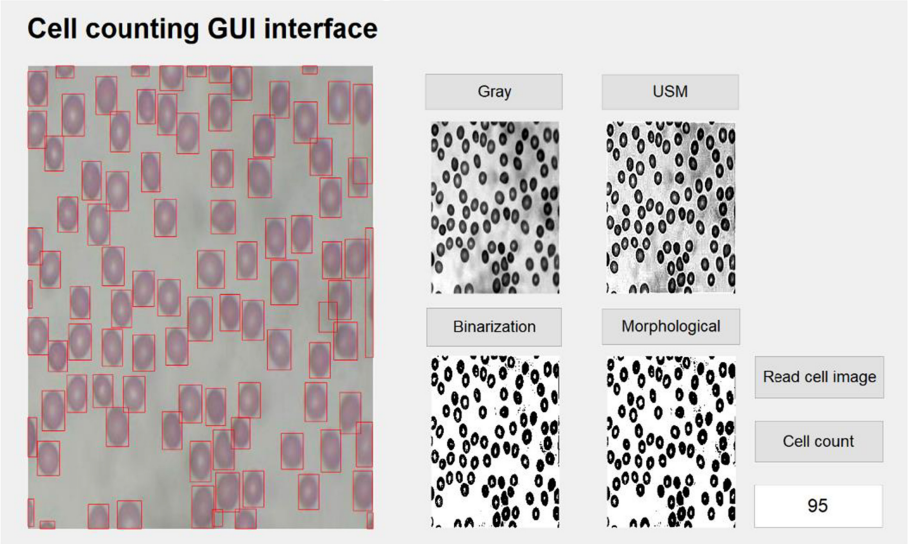


Fig. 1. GUI of cell counting

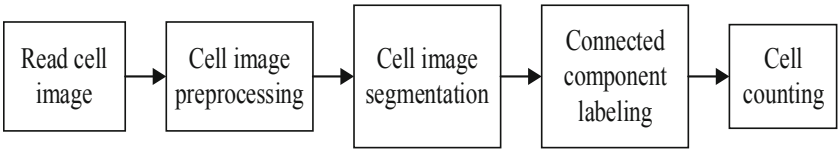


Fig. 2. Flow chart of cell counting

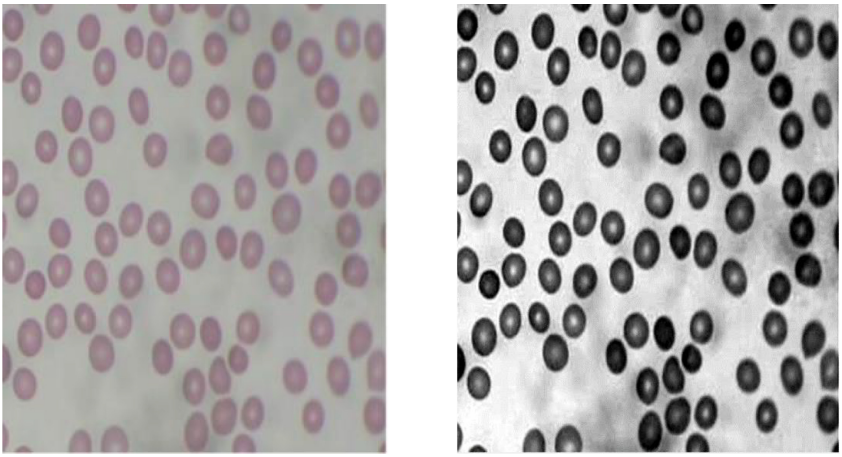
2 Cell Image Preprocessing

2.1 Gray

The cell microimage is an unprocessed color RGB image. In order to reduce the computational burden of image processing, this paper adopts the weighted average method for grayscale [11] processing, as shown in formula (1):

$$Gray = [R \quad G \quad B] \begin{bmatrix} 0.299 \\ 0.287 \\ 0.114 \end{bmatrix} \tag{1}$$

The image after graying processing is shown in Fig. 3.



(a) Original image (b) Grayscale image

Fig. 3. Image graying processing

2.2 USM Sharpening Processing

Sharpness [12] is an attribute of image detail sharpness. In this paper, cell edge contrast was enhanced by sharpening the cell image. There is a big difference between the gray level of the target edge and that of the background, so the global binarization can be carried out better. The USM sharpening process is shown in Fig. 4:

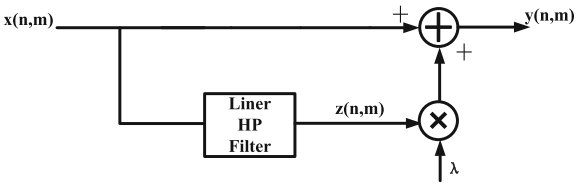
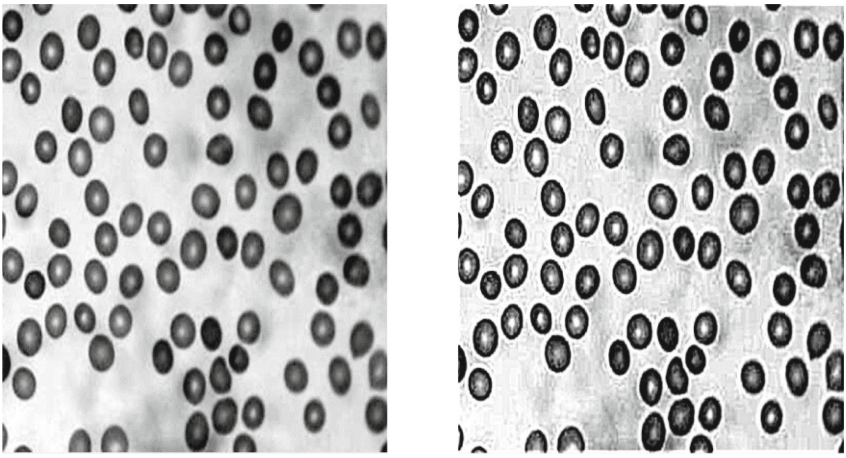


Fig. 4. USM model

The specific formula is expressed as Eq. (2):

$$y(n,m) = x(n,m) + \lambda z(n,m) \tag{2}$$

In the formula, $x(n,m)$ is the input image, $y(n,m)$ is the output image, $z(n,m)$ is the correction signal, which is obtained by high-pass filtering the original image, and λ is the scaling factor used to control the enhancement effect. The USM sharpening result of the cell image is shown in Fig. 5.



(a) Grayscale image (b)USM sharpening image

Fig. 5. USM sharpening process

3 Cell Segmentation

3.1 Binarization

In order to get the cell image from the background image better, the grayscale image needs to be binarized. Binarization can be divided into global binarization and adaptive binarization. The global binarization method has a very good effect on simple image processing of picture information. An image $f(x,y)$ consists of background pixels and target pixels. If you want to extract the target image from the background, you need to find the optimal threshold T. Make all the points (x,y) of $f(x,y) \leq T$ the target points, and vice versa as background points. The segmented image is shown in formula (3).

$$g(x,y) = \begin{cases} 1, & f(x,y) > T \\ 0, & f(x,y) \leq T \end{cases} \tag{3}$$

In this paper, the Ostu method [13, 14] is used to calculate the optimal threshold. The implementation principle is as follows:

1) Normalized histogram (Gray level L, total pixels N, probability of occurrence of each grayscale pixel is p_i)

$$N = \sum_{i=0}^{L-1} n_i, p_i = \frac{n_i}{N} \tag{4}$$

2) Calculate the occurrence probability of background pixel (p_A) and target pixel (p_B)

$$P_A = \sum_{i=0}^{t} p_i, P_B = \sum_{i=t+1}^{L-1} p_i = 1 - p_A \tag{5}$$

3) Calculate the inter-class variances of the background and target regions

$$\mu_A = \frac{\sum_{i=0}^{t} ip_i}{p_A}, \mu_B = \frac{\sum_{i=t+1}^{L-1} ip_i}{p_B} \tag{6}$$

$$\mu_0 = p_A\mu_A + p_B\mu_B = \sum_{i=1}^{L} ip_i \tag{7}$$

$$\sigma^2(t) = p_A(\mu_A - \mu_0)^2 + p_B(\mu_B - \mu_0)^2 \tag{8}$$

In the formula, μ_A and μ_B represent the average gray value of the target image and background respectively, and μ_0 represents the average gray value of the whole image. The image after binarization is shown in Fig. 6.

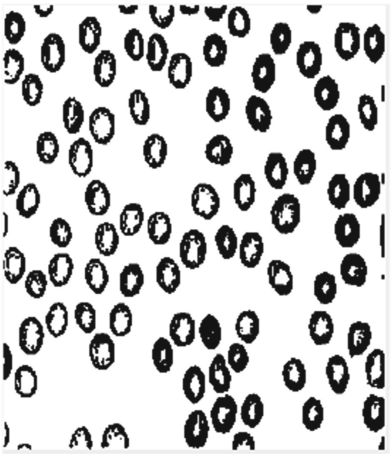


Fig. 6. Binarization image

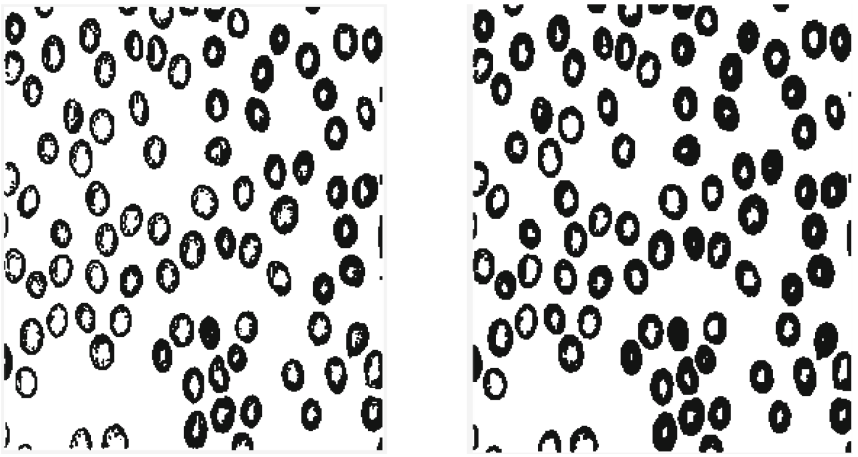
3.2 Morphological Treatment

After image binarization, there will still be problems such as more corner burrs, cell. adhesion, extremely small noise points, and small holes. The open operation of morphological image processing [15–17] can solve these problems well. The open operation first performs the corrosion operation on the image, and then uses the same size structure element to perform the expansion operation on the corroded image.

The open operation of structure element B on set A, expressed as $A \circ B$, and its definition is shown in formula (9):

$$A \circ B = (A \Theta B) \oplus B \tag{9}$$

The cell adhesion is less after open operation, and the small boundary cavity is filled well. To further optimize the segmentation effect, the median filter is used to remove the minimal noise points, and finally a relatively ideal cell microsegmentation image is obtained, as shown in Fig. 7.



(a) Image before morphological processing (b) Image after morphological processing

Fig. 7. Comparison before and after morphological treatment

4 Cell Counting

After morphological processing of binary images, an ideal segmentation image with clear cell edges, cavity filling and fewer adhesion areas is obtained to facilitate the following cell count statistics. Connected component labeling algorithm [18–20] is a common method for binary image analysis, which can be used to extract the features of the target in the image, The schematic diagram is shown in Fig. 8.

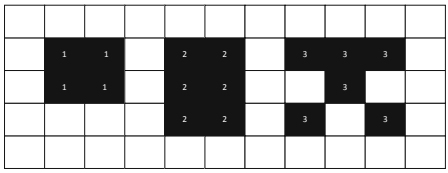


Fig. 8. Connected domain marking

In this paper, cell counting is achieved by counting the number of connected domains.

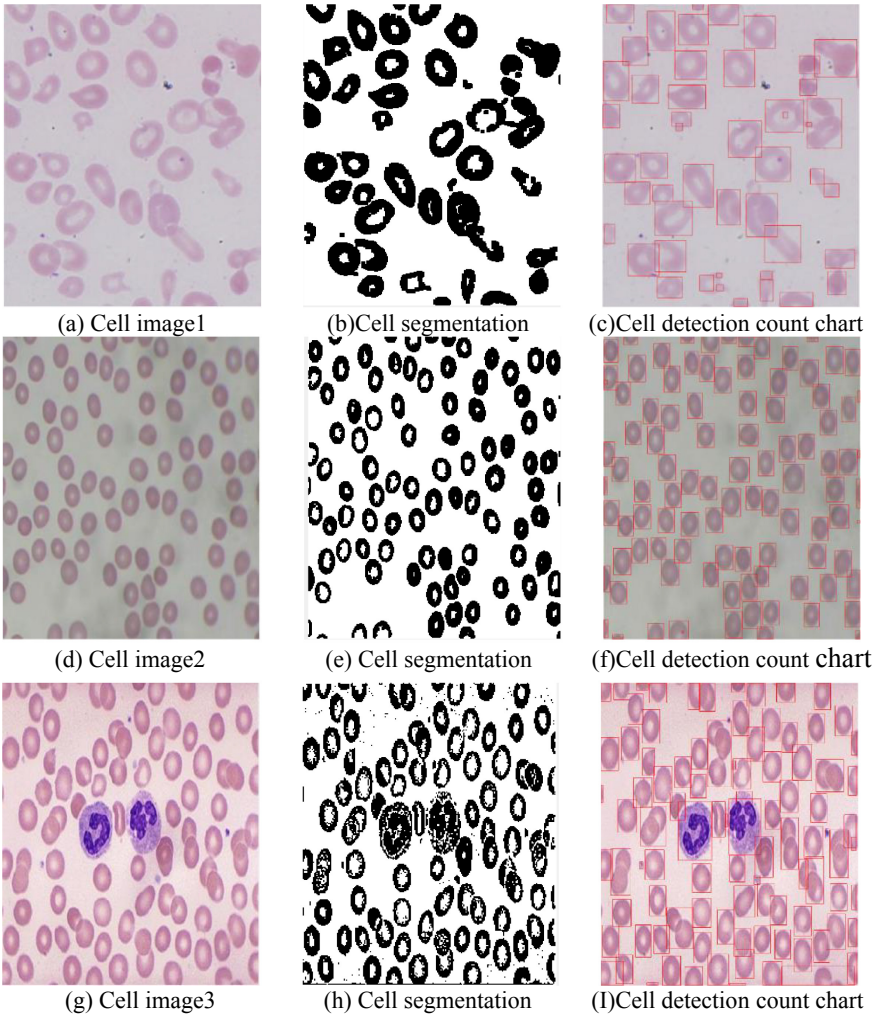
in a binary image. Taking 8-connected labeling as an example, the principle is:

1) Traverse the whole image, when the target pixel $f(x, y)$ is scanned, judge whether the pixel is marked, if the pixel point is not marked, save its coordinate value $M[x][y]$ to the queue, And mark the pixel point at the corresponding coordinate position of the marking matrix.
2) Scan 8 neighborhoods of pixel point $f(x, y)$. When the new unmarked target pixel point is scanned, the coordinate value $M[x+1][y]$ of the point $f(x+1, y)$ is saved in the queue and marked in the marking matrix.
3) When the 8 neighborhood scan marks are completed, $f(x, y)$ is listed, and the column head is $f(x+1, y)$. Then the 8-neighborhood scan and marks in Step 2 are performed again.
4) When a connected area is marked, the mark count is incremented by 1, the queue is cleared, and steps 1 to 3 are performed again to mark the new connected area.
5) After traversing the complete picture, the number of connected domain markers is the number of cells.

5 Experimental Results and Analysis

In this paper, the stained red blood cells is taken as the research object, and part of the original images is intercepted for image processing and counting,as shown in Fig. 9.

In order to verify the feasibility of the method in this paper, the statistical results are compared with the manual counting results. The comparison results are shown in Table 1. It can be seen from Table 1 that the method in this paper can count the number of cells well, whether it is a sparse cell image or a dense cell image. The results show that this method has low counting error and strong versatility.



(a) Cell image1 (b)Cell segmentation (c)Cell detection count chart

(d) Cell image2 (e) Cell segmentation (f)Cell detection count chart

(g) Cell image3 (h) Cell segmentation (I)Cell detection count chart

Fig. 9. Experimental results

Table 1. Cell counting error table

Classifly	Cell image1	Cell image2	Cell image3
Manual counting results	43	93	100
System counting results	45	95	95
Absolute error	4%	2%	5%

6 Conclusions

This paper presents a method for cell counting based on Matlab language.This method firstly increases the contrast between the object and the background by USM sharpening the grayscale image, and then performs global threshold segmentation and mathematical morphology processing to obtain the microcell segmentation image with clear edge contour and less target adhesion.Finally, the cells are quickly labeled and counted. The results show that the method has strong adaptability and low counting error.The next step is to study the isolation of overlapping cells to improve the reliability of counting.

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