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A semi-automatic method for robust and efficient identification of neighboring muscle cells

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A R T I C L E I N F O

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A B S T R A C T

Segmentation and identification of muscle cells robustly and efficiently is of considerable importance in determining the muscle's physiological conditions. It is challenging due to frequently occurring artifacts, indistinct boundary between adjacent cells, the arbitrary shape and large number of cells. Currently, the widely used segmentation and quantification tools are usually manual or semi-automatic, which is time-consuming and labor intensive. In this paper, a semi-automatic method is proposed to segment the muscle cells robustly and efficiently. The proposed approach utilizes and evolves three fundamental image processing techniques, threshold selection, morphological ultimate erosion and morphological dilation. Experimental results verified the effectiveness of the proposed method.

1. Introduction

Image segmentation [1–4] plays important roles in many computer vision applications. With the rapid development of microscopy imaging [5], many new scientific applications are brought forth in the areas of physics, chemistry and biology [6,7]. Among them, cell segmentation is the most studied and popular one. Many methods were proposed and claimed to be superior in segmenting a class of cell images, e.g. watershed method [8], region growing method [9], morphological method [10], neural network method [11], graph method [12] and machine learning and modeling method [13,14]. In this paper, we propose a method to segment the muscle cell/fiber image which usually contains a lot of cells/fibers and would cost great labor and time for manual or semi-automatic methods. Hence, image processing techniques that is capable of segmenting the neighboring cells and identifying their boundaries robustly and efficiently becomes very important. Past efforts in segmenting the muscle cell/fiber images include: (1), active contour based method [15,16,18]; (2), GVF method [17]. Experimental results indicate that our proposed method is superior to them in efficiency and accuracy.

There are two general properties of the muscle cell images, intensity and intensity gradient and they are identified mainly by their intensity gradients and local intensities. It is difficult for one to notice the global intensity variations of these images accurately because of lateral inhibition. Hence, we enhance the boundary of all the cells and use the segmented intensity gradients as constraint for the segmentation with a global threshold. To segment the enhanced gradient image robustly, we calculate the threshold based on the slope variation of the histogram distribution [19]. The threshold is selected as the point with greatest variation, which is more accurate than the state of art threshold selection methods [20–34]. After the image was segmented by the selected threshold, some neighboring cells are still connected. Thus, further processing is needed to separate them from each other. To separate them, we propose an iterative erosion method based on an area threshold that can distinguish the area of the cell and the area of the noise which is prevalent in image processing applications [35]. The area threshold is computed on line. After the cell is identified, its boundary is delineated by dilating the segmented seed toward its edge until part of it reaches the edge.

2. The proposed method

2.1. Segmentation by threshold selection

The segmentation comprises three parts. The first part is segmentation of the original image by a global threshold. The second part is producing the gradient image and segmenting it by a global threshold. The third part is combining the first part and second part for the final segmentation based on the characteristics of the muscle cell images.

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For the first part of segmentation, we need to compute a threshold from the original image. An improved threshold selection method is proposed in this paper based on our previous study [15]. The histogram distribution of the original image is calculated and then transformed into the Fourier domain. The first term of the Fourier transform corresponds to the DC value of the histogram distribution. The second term of the Fourier transform corresponds to the sketchiest global information of the histogram distribution. Suppose the image contains the object and the background and their gray-scales are Gaussian distributed, the second term of Fourier transform corresponds to a sketchy overlay of two Gaussian distributions. Hence, a global variation peak exists between these two Gaussian distributions to separate them roughly. This global variation peak is detected automatically and it is then used as the benchmark threshold to segment the original image. The threshold selection method contains the following steps:

Step 1: Re-arrange the gray-scale values of the image in the interval [1, 255] and compute its normalized histogram distribution, $P(x)$:

$$P(x=i)=\frac{N_i}{N_j};i=1,2,...,255 \tag{1}$$

$$j=\underset{k\in[1\quad 255]}{\operatorname{argmax}}\{N_k\} \tag{2}$$

where N_i denotes the frequency of the gray-scale i and N_j denotes the maximum frequency which occurs at j in the interval $[1\quad 255]$.

Step 2: Transform $P(x)$ by the Discrete Fourier Transform (DFT):

$$F(k)=\sum_{x=1}^{255}P(x)e^{-i\frac{2\pi kx}{255}};k=1,2,...,255 \tag{3}$$

Step 3: After DFT, choose the first term and second term of the low frequency parts and eliminate the other parts by the following equation:

$$F'(k)=\begin{cases}F(k);k=1,2;\\F(k);k=254,255;\\0;k=3,\ldots,253\end{cases} \tag{4}$$

Step 4: Transform from the frequency domain back into spatial domain to get the smoothed histogram.

$$P'(x)=\frac{1}{\mathrm{T}}\sum_{k=1}^{255}F'(k)e^{i\frac{2\pi xk}{255}};x=1,\ldots,255 \tag{5}$$

Step 5: There are two slopes for each point on $P'(x)$, one on the left and the other on the right. They are computed by fitting a line model with 15 adjacent points at each side. All the reasonable numbers from 3 to 81 are tested on a variety of images and it is found that the numbers from 7 to 21 are the most effective parameters for the histogram range from 1 to 255. So we choose 15 for all the experiments. The line model is formulated as:

$$y_i=ax_i+b \tag{6}$$

$$[a,b]^T=\left(B^TB\right)^{-1}B^TY \tag{7}$$

$$B=\begin{bmatrix}x_1 & 1\\x_2 & 1\\\vdots & \vdots\\x_{15} & 1\end{bmatrix} \tag{8}$$

$$Y=\quad[y_1,y_2,\ldots,y_{15}]^T \tag{9}$$

Two slopes at point i, $a_1(i)$ and a_2 (i), are then obtained from Eq. (7).

Step 6: Compute the slope difference, $s(i)$, at point i:

$$s(i)=a_2(i)-a_1(i);i=16,17,...,240 \tag{10}$$

Find the positions in the slope difference distribution that meet the following conditions:

$$s(i)-s(i-1)=0 \tag{11}$$

We get the valleys $V_i;i=1,2,...,N_v$ with greatest local variations and the peaks $P_i;i=1,2,...,N_p$ with greatest local variations of the slope difference distribution. Suppose there are two histogram peaks, P_1 and P_2 corresponding to the centers of the two Gaussian distributions, we choose the position where the valley V_i between P_1 and P_2 as the benchmark threshold T_b. Then the original image, I is binarized as follows:

$$S_I=\begin{cases}1;I\geq T_b\\0;I<T_b\end{cases} \tag{12}$$

For the second part of segmentation, we need to form the gradient image from the original image. The Sobel operator is used to detect the intensity gradient in the row direction and column direction respectively and get two gradient images I_x and I_y . Then the gradient magnitude image is computed as follows:

$$I_g(k,j)=\sqrt{I_x{}^2(k,j)+I_y{}^2(k,j)} \tag{13}$$

where (k,j) denotes the index of the pixel position.

The gradient image is then segmented with a global threshold to obtain the edge image, S_E. We need the segmented edges to be as complete as possible for the subsequent processing. Unfortunately, none of the state of art threshold selection methods [20–34] could yield acceptable accuracy. Hence, we use the same criteria to select the threshold as [19] and it contains the following steps.

Immediately following the first two steps, **Step 1** and **Step 2** described above.

Step 7: Choose the low frequency parts and eliminate the high frequency parts by the following equation.

$$F'(k)=\begin{cases}F(k);k=1,2,\ldots,10;\\F(k);k=246,\ldots,254,255;\\0;k=11,\ldots,245\end{cases} \tag{14}$$

k is chosen from 1 to 10 to keep more details of the image. As a result, the selected threshold could segment the image and keep the edge more complete. Transform from the frequency domain back into spatial domain by the following equation.

$$P'(x)=\frac{1}{\mathrm{T}}\sum_{k=1}^{255}F'(k)e^{i\frac{2\pi xk}{255}};x=1,\ldots,255 \tag{15}$$

$P'(x)$ is the smoothed histogram distribution.

Step 8: Repeat the above **Step 5** and **Step 6**. We get the valleys $V_i;i=1,2,...,N_v$ of the slope difference distribution with local variation peaks. There are valleys adjacent to the benchmark threshold T_b, some on the left and the others on the right. The valley on the left is selected to make the segmented edge to be as complete as possible. Accordingly, the gray-scale value corresponding to the left valley is selected as the optimal threshold, T_o.

The edge image is obtained by the following equation.

$$S_E=\begin{cases}1;I_g\geq T_o\\0;I_g<T_o\end{cases} \tag{16}$$

With the two segmentation results, S_E and S_I, we calculate the final segmentation S_f in two cases due to the vast variety of muscle cell types. The user decides which case to choose for one specific type of muscle cell images. There are muscle cell images with