EFFECTIVE APOPTOTIC CELL EXTRACTION FROM VIDEO MICROSCOPY IMAGES

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ABSTRACT

This paper presents an automatic extraction method for apoptotic cells from video microscopy images. Apoptosis, which is also called programmed cell death, is well-known to be closely related with various diseases. Detection of apoptotic cells can help scientists to study apoptosis and to reveal its mechanism, so that new medical technologies can be exploited. Scientists often analyze the images acquired with video microscopes with some sort of image analysis tools. However, it is usual that the performance of the tools is not enough to thoroughly detect the apoptotic cells, thus the researchers have to manually detect the apoptotic cells from the images. This paper proposes a method to automatically detect the apoptotic cells based on their inherent shape features. The proposed method is applied to real world video microscopy images containing apoptotic cells and the results demonstrate its high performance to successfully extract the cells.

1. INTRODUCTION

Recently, much attention has been paid to apoptosis in the field of pathology. Apoptosis, which is called programmed cell death, is a process of the pattern of events during cell death, and it is well-known to be a significant component controlling health of multicellular organisms and a cause for serious diseases, such as cancer and AIDS. In order to develop the medical care of these diseases, lots of researchers try to reveal the mechanism of apoptosis[1] by observing the pattern of events of the cells in video microscopy images. Video microscopy images are digitally acquired with a video microscope which consists of an optical microscope and a CCD camera[2]. The researchers usually analyze the video microscopy images by using simple software or visual recognition, and thus they have to make considerable manual labor. Several image analysis methods for other types of objective have been proposed[5, 6], however, they cannot be applied to the apoptotic cell extraction, because the shape features are different. Taking into account of the intrinsic shape features of the apoptotic cells, this paper proposes an effective automatic extraction method for the apoptotic cells from the video microscopy images.

Experiments for extraction of apoptotic cells from real world video microscopy images show the high performance of the proposed method.

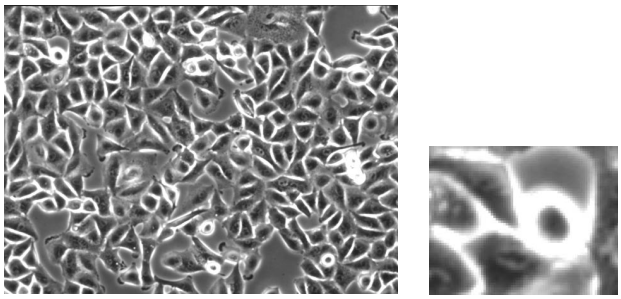

Fig. 1. (a) An observed image with size of 624×480 pixels and 8 bits/pixel gray levels, (b) an example of the apoptotic cells in (a).

2. FEATURES OF THE APOPTOTIC CELLS

For the analysis of the apoptosis mechanism, we acquire video microscopy images of *A549* [1] with some enzymes[2]. One frame of the 256-gray-level images is shown in Fig. 1(a), and an apoptotic cell in Fig. 1 (a) is also shown in Fig. 1(b). Based on the knowledge of the apoptotic cells and as shown in Fig. 1(b), the apoptotic cell can be characterized by the following three significant shape features:

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[1] *A549*; The lung carcinomatous tissue of human.

[2] *TNF-*α and luciferase; *TNF-*α is a tumor necrosis factor, and luciferase is a type of protein.

***Feature*1)** The apoptotic cell has a quasi-circular form with much higher gray level than the other cells. Further, the gray level in the central area is much lower than its contour's.

***Feature*2)** The contour of the apoptotic cell is thicker than the contours of the other cells.

***Feature*3)** No overlapping cells exist.

The above *Feature*'s are inherent to the apoptotic cells, thus they are fully utilized in the proposed method for the extraction of the apoptotic cells.

3. AUTOMATIC EXTRACTION METHOD OF THE APOPTOTIC CELLS

The proposed method consists of the following three systems:

***System*1: Extraction of Processing Target Regions**
According to *Feature1*, the quasi-circular contours are extracted from the binary image of the observed image which is obtained by a threshold. There is a strong possibility that the apoptotic cell exists in the region including the extracted contour. This region is called the Processing target region hereafter.

***System*2: Shape Analysis and Cell Extraction**
The Processing target regions are analyzed based on a new shape parameter. According to the analysis results, the apoptotic cells can be extracted.

***System*3: Verification by Moving Pictures**
Since the video microscopy images are acquired as moving pictures containing many frames recording the changes of the organ tissue over time, according to the life span of the apoptotic cells, the detection results of the current frame can be enhanced by using the detection results of previous frames, and thus more accurate extraction can be obtained.

More details of each system are given in the following subsections.

3.1. System1: Extraction of the Processing Target Region

Procedure1: The binary image f_b is obtained by processing the observed image f_o with a predefined threshold T_{binary}. For example, the binary image of Fig. 1 (a) is shown in Fig. 2 (a), where $T_{binary} = 0.8G$ (G: the maximum gray level of f_o). Further, $f_b(i,j) \in \{1,0\}$ and $f_o(i,j)$ $(i = 1,\ldots,M; j = 1,\ldots,N)$ denote the pixel values at (i,j) of f_b and f_o, respectively.

Procedure2: A thinning method[3] is applied to the binary image f_b and then obtain the image f_t.

Procedure3: For extraction of the contour satisfying *Feature1*, Hough transform[4] is applied to f_t.

Procedure4: The contours which large votes are polled for in Hough transform are selected, which is a quasi-circle by strong possibility. According to *Feature3*, overlapped circles among the selected ones are eliminated. Then the vote counts of the eliminated circles are added to the vote count of the remaining circle. If the vote of the remaining circle is more than Th_v, where Th_v is a threshold, it is extracted for the next procedure.

procedure5: The area enclosed in a circle with double the radius of the circle extracted in Procedure 4, which is called the Processing target region. An example image indicating the Processing target regions, which are obtained from Fig. 2 (a), is shown in Fig. 2 (b).

The next subsection explains the shape analysis performed in the Processing target regions.

3.2. System2: Shape Analysis and Extraction of Cells

The Processing target region is denoted by $R_k (k = 1,\ldots,N;$ N is total number of the Processing target regions), and f_b in R_k is denoted as $f_b^{R_k}$. A new parameter P_f, which is used for the shape analysis, is defined below.

$$P_f = \sqrt{(M_n)^2 + (S_r)^{-2}} \tag{1}$$

where

$$\begin{aligned} M_n &= \frac{2\pi\,(M_{02} + M_{20})}{M_{00}^2} \\ M_{pq} &\triangleq \sum_{(i,j)\in R_k} \{(i - i_G)^p (j - j_G)^q\}\, f_b^{R_k}(i,j) \\ S_r &\triangleq \frac{4\pi M_{00}}{L^2} \quad , \end{aligned}$$

(i_G, j_G) represents the gravity of $f_b^{R_k}$, and L is the length of the contour.

Let us explain how to judge whether the area R_k includes the apoptotic cell by using Eq. (1). Based on the definition in Eq. 1, the following can be recognized:

(i) When $P_f = \sqrt{2}$, the contour included in R_k is a perfect circle.

(ii) When $P_f \simeq \sqrt{2}$, the contour is similar to the circle.

(iii) When $P_f >> \sqrt{2}$, the contour is not a circle definitely.

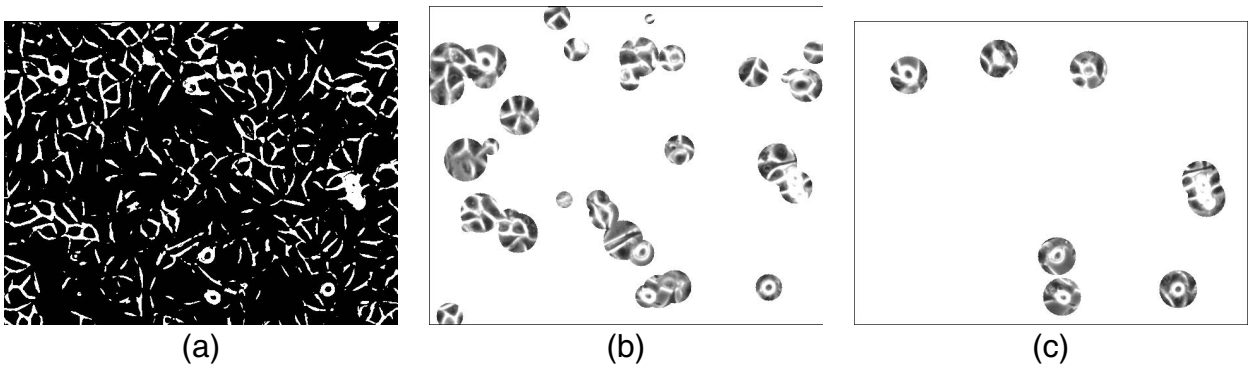

Fig. 2. Explanation of *System1* and *System2*: (a) The binary image f_b of Fig. 1 (a) obtained by Procedure1 of *System1*; (b) Processing target regions obtained from (a) by Procedure5 of *System1*; (c) The apoptotic cells extracted from (b) by *System2*.

Therefore, since the apoptotic cell is a quasi-circle as described in *Feature1*; by selecting the processing target regions R_k's with $P_f \simeq \sqrt{2}$, we can extract the apoptotic cells. Actually in order to judge whether $P_f \simeq \sqrt{2}$, we use the following equation:

$$P_f \leq T_{P_f}, \tag{2}$$

where T_{P_f} is a predefined threshold.

Further, before computing P_f, we have to determine the gravity of $f_b^{R_k}(i_G, j_G)$. Since the contour is not a complete circle and includes some of the contour of other neighbor cells, the correct gravity of the contour of only the apoptotic cell cannot be calculated by traditional gravity computation manners. Therefore, we compute the gravity by using the contour pixels polling votes in Hough transform as a circle in Procedure3 of *System1*. Owning to this modification, we can determine the gravity located in the center of the apoptotic cell, and thus the P_f computed by the gravity can provide the accurate extraction results. As an example, the extraction result from Fig. 2 (b) is shown in Fig. 2 (c).

3.3. System3: Verification by Moving Pictures

Since the video microscopy images are normally acquired as moving pictures over a time period, the detection results obtained by *System1* and *System2* for the current frame can be refined by comparing with the detected results of the previous frames, and more accurate results can be obtained. For example, an over-detected cell included in the current frame can be eliminated if any extraction cells correspond to the cell do not appear in the neighbor frames.

4. EXPERIMENTAL RESULTS

Some experiments are performed in order to verify and examine the high performance of the proposed method. It

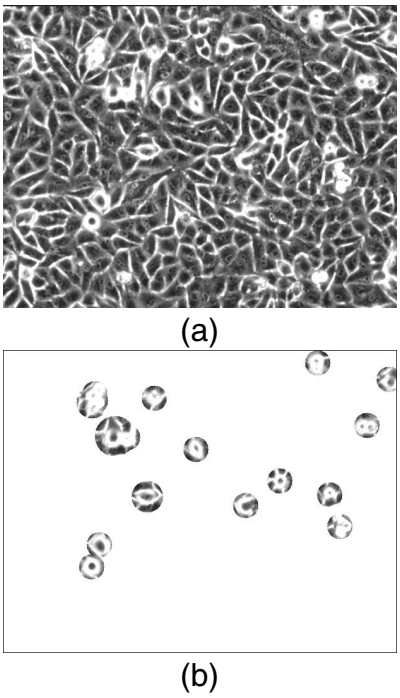

Fig. 3. Experimental Results 1: (a) One frame of the observed microscopy images; (b) Extraction results obtained by applying *System1* and *System2* to (a). This result shows a complete extraction of the apoptotic cells in the frame without under- or over-detection even without *System3*.

is applied to the video microscopy images of *A549*, which contains 343 frames acquired as moving pictures. First, one of the frames is shown in Fig. 3 (a). *System1* and *System2* are executed for this frame, and the extraction result is shown in Fig. 3 (b). The parameters are $T_{binary} = 0.8G$, $Th_v = 10$, and $T_{P_f} = 4.0$, respectively. As shown in Fig. 3 (b), there are no un-detected or over-detected apoptotic cells

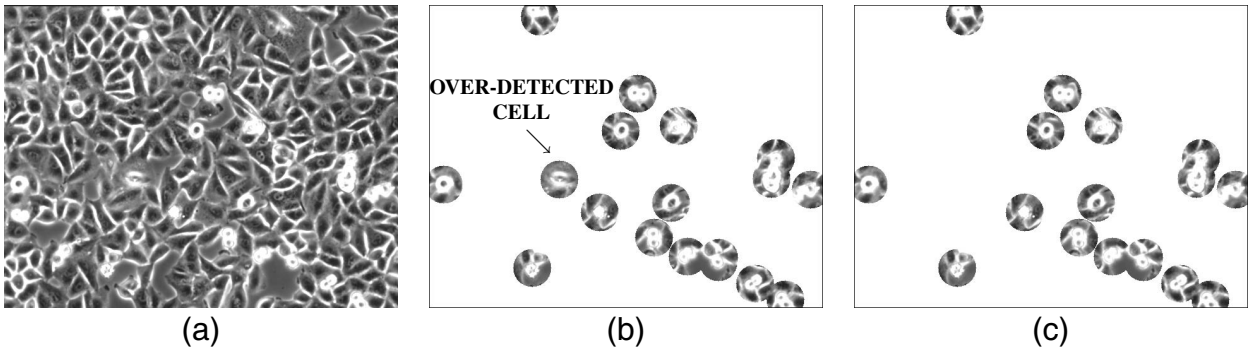

Fig. 4. Experimental Results 2: (a) A frame of the observed microscopy images; (b) Extraction results by applying *System1* and *System2* to (a), there is one over-detected cell existing; (c) The over-detected cell is eliminated when applying *System3*.

in the extraction results without *System3*.

Next, another extraction experiment for a different frame is shown in Fig. 4. *System1* and *System2* are applied to (a), and the extraction result is Fig. 4 (b). The parameters T_{binary}, Th_v, and T_{P_f} are the same as in the previous experiment. There is one un-detected cell as indicated in Fig. 4 (b). By further applying *System3*, we can obtain the results as shown in Fig. 4 (c), in which the over-detected cells is eliminated.

Finally, all 343 frames of the images are processed by the proposed method, and its results are summarized in Table 1. According to Table 1, for all images, (a) with *System1* and *System2*, there is no any un-detected apoptotic cells, but there is a 2% of the frames have over-detected cells; (b) with all of three systems of the proposed method, the detection results are 100% with neither un-detected cells nor over-detected cells.

Consequently, we can see that the proposed method can accurately extract the apoptotic cells from the images. Fig. 4 (b)

Table 1. The performance of the proposed method for all 343 frames of the video microscopy images: (a) the results by execution of *System1* and *System2*; and (b) the results by execution of all three systems.

	(a)	
(i)	The frames including un-detected cells	0%
(ii)	The frames including over-detected cells	2%
	(b)	
(iii)	The frames including un-detected cells	0%
(iv)	The frames including over-detected cells	0%

5. CONCLUSIONS

This paper proposes an automatic extraction method for the apoptotic cells from video microscopy images based on the intrinsic shape features of the apoptotic cells. The experimental results demonstrate that the proposed method can effectively extract the apoptotic cells, and the success extraction ratio can reach 100%. The proposed method thus can help for analyzing the video microscopy images for study the apoptosis.

6. REFERENCES

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