Apoptosis 세포의 자동화된 분할 및 인식을 위한 강인한 방법

(A Robust Method for Automatic Segmentation and Recognition of Apoptosis Cell)

류 해 릉 † 신 영 숙 ††
(Liu, Hai-Ling) (Young-Suk Shin)

요 약 본 연구는 Apoptosis세포들의 형상을 검출하기 위하여 전통적인 세포측정법과는 다른 영상기반 접근법을 제안한다. 이 방법은 세포측정법의 단점을 극복하고 Apoptosis 세포들을 정확하게 인식할 수 있다. 본 연구에서 K-means 군집화 방법이 Apoptosis 세포의 거시적인 분할을 행하는 데 사용되었으며, '스네이크'라고 불리는 액티브 윤곽선 모델이 정밀한 경계선 검출을 위해 사용되었다. 그리고 Apoptosis세포들의 물리적 특징, 형태적 특징 그리고 무늬특징들을 포함하는 몇가지 특징들이 추출되었다. 마지막으로 Mahalanobis 거리 분류기가 Apoptosis세포와 비 Apoptosis 세포로서 분할영상들을 분류한다.

키워드 : 에이팝토시스, 분할, 액티브 컨투어

Abstract In this paper we propose an image-based approach, which is different from the traditional flow cytometric method to detect shape of apoptosis cells. This method can overcome the defects of cytometry and give precise recognition of apoptosis cells. In this work K-means clustering was used to do the rough segmentation and an active contour model, called 'snake' was used to do the precise edge detection. And then some features were extracted including physical feature, shape descriptor and texture features of the apoptosis cells. Finally a Mahalanobis distance classifier classifies the segmentation images as apoptosis and non-apoptosis cell.

Key words : apoptosis, segmentation, active contours

1. 서 론

Apoptosis is a specific mode of programmed cell death in multi-cellular organism. Cells undergoing apoptosis in normal and neoplastic tissues display, the following morphological and biochemical features: chromatin condensation, fragmentation of the nucleus, cytoplasmic retraction and finally, loss of membrane integrity as well as production of apoptotic bodies containing intact organelles and plasma membrane [1-4]. Many of the anticancer medicines currently used act through the activation of cell elimination by inducing active cell death.

Flow cytometric method(FCM) is most frequently used to recognize apoptosis[5,6]. FCM analysis is performed with a cytometer equipped with an argon laser. When particle streams pass through the light emitted by the laser, they will scatter the light in some way and fluorescent chemicals found in the particles or attached to the particles may be excited into emitting light at a higher wavelength than the light source. Detectors pick up the combination of scattered and fluorescent light and the computer calculates the fluorescence ratios, which are incremented in a frequency histogram. A list of physical and chemical characteristics, or parameters, of cells can be measured by flow cytometry[7]. FCM allowed us to detect and separate apoptotic cells from normal cells simultaneously and to analyze the position in the cell cycle of each cell population identified. But FCM has two remarkable drawbacks: (1) The reliability of FCM based on the scattered light is strongly influenced by the uniform of the shape of the cells; (2) Cell death results in an immediate decrease of scatter signals. So in our works we detected the apoptosis using a scan of a color photograph of stained cancer cells from a microscope.

· The authors wish to acknowledge the assistance and support of Cancer Treatment Center of Chosun University, Korea.
· 이 논문은 제34회 추계학술대회에서 'Apoptosis 세포의 자동화된 분할 및 인식을 위한 강인한 방법'의 제목으로 발표된 논문을 확장한 것임

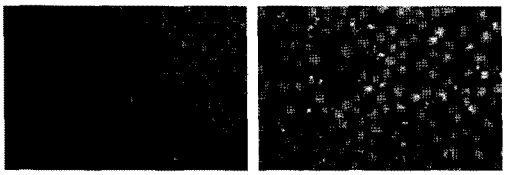
† 비 회 원 : 조선대학교 정보통신공학과
allen1979_12@hotmail.com
†† 정 회 원 : 조선대학교 정보통신공학과 교수
ysshin@chosun.ac.kr
(Corresponding author임)

논문접수 : 2008년 12월 22일
심사완료 : 2009년 4월 20일

2. Methods

2.1 Material and main procedure

HepG2 human hepatoma cells that we use in our experiments are from a 14 years old boy with hepatocellular carcinoma, provided by Cancer Treatment Center of Chosun University, Korea. All chemicals used are of the highest purity with commercially use. The cancer cell specimens are stained with Hoechst 33342(H342). H342 is a kind of fluorescent stains for labelling DNA in fluorescence microscopy, which is more lipophilic, and thus more able to cross the intact cell membrane and stain nucleolus. A remarkable feature is that the fluorescence intensity of H342 in apoptosis cell is stronger than that in normal cells. Different patterns are showed in Fig. 1.



(a) (b)

Fig. 1 Different statuses of normal and apoptotic cells stained by H342: (a) Normal cells (b) Apoptotic cells

From a comparison between Fig. 1(a) and (b), we can see that the fluorescence intensity of apoptosis is far stronger than that in normal cells and apoptosis is quite different from normal cells in shape, texture etc. A fragmentation of the nucleus and apoptotic bodies shows in some apoptosis cells. The methodology in our paper is based on standard and well-known algorithms including histogram segmentation, active contour model, and gray lever occurrence matrices. The additional steps in Fig. 2 are required to discriminate the apoptosis cells.

2.2 Preprocessing

It is usually very hard to get photographs of biopsies of good quality. The main reason is caused by the problem of stain and focusing of microscope. For example, the same kind of image was stained very differently with various hue and saturation values. At this time, a preprocessing is required to normalize preliminarily the images by matching each photograph against a representative one[8]. We noticed that the true-color space does not return an any particular information[9]; therefore we have preferred representing the images in the luminance channel (V) of the HSV space[10].

A simple adaptive self-tuning technique, a generalized method of Otsu(1979), has been developed to detect the optimal thresholding values[11]. But Otsu's method doesn't work well with variable illumination and is not suitable to the unevenly stained images. Therefore, in our work we adopted K-means algorithm, a clustering method, which minimizes the sum of the within-cluster sums in point-to-cluster-centroid distances. We iteratively apply a K-means clustering algorithm on the histogram of the whole image with the gray level=80 and T_2=196 respectively from the centroid of background and foreground. This process usually converges after a few iterations and as T_1 and T_2 become stably, that leads to a rough segmentation of cells(Fig. 3).

In the result image, we can see that K-means method can obtain more detailed image and is more

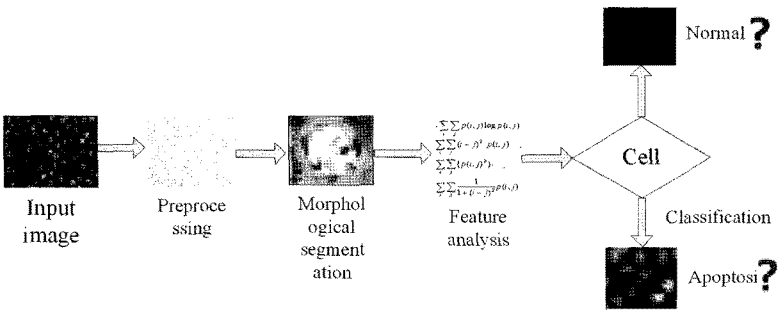


Fig. 2 Flow diagram of processing

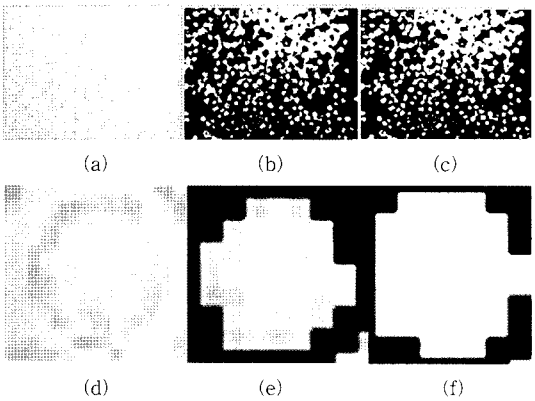


Fig. 3 The comparison of rough segmentation between K-means method and Otsu's method: (a) HSV image (b) a rough segmentation result of K-means (c) a segmentation result of Otsu's method (d) an enlarged image of one cell (e) an enlarged segmentation result of K-means method (f) a enlarged segmentation result of Otsu's method.

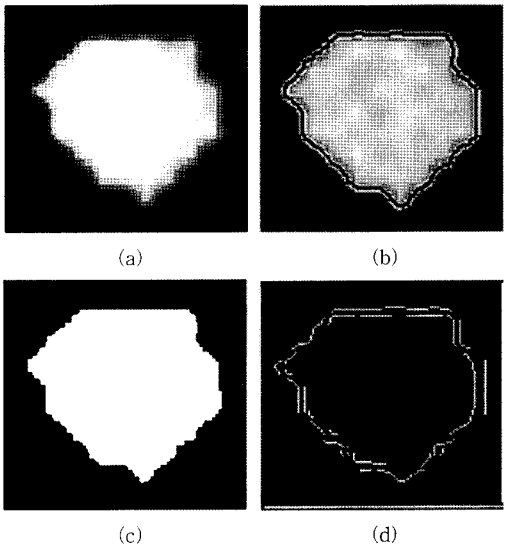


Fig. 4 Edge detection results: (a) Original HSV image (b) Edge detection result after iterations (c) Global result (d) Edge detection result by Canny

similar to the original image than Otsu's method.

2.3 Edge detection

The precise edge detection of cell nuclei is the most demanding step in the whole processing steps. The manual corrections are tedious and are thus out of the question in large-scale clinical use. The traditional methods such as Canny edge detecting will produce lots of spurious edges or the edges are not continuous.

A contour-based approach is especially preferred against the method of traditional edge detection for smooth membranes like cell nuclei. A segmentation method that lets a contour model iteratively converge toward an underlying image feature is called active contour or snake[12]. A movement is driven by external image forces, usually derived from image edges, and internal forces that can model features like stiffness or tensibility. Because of these simulated physical features the method is known to be robust against noisy or partially missing image information. Although the method doesn't detect well for concave and convex, it detects very well for the shape like circular cells.

The original snake model proposed by Kass[13] is restricted by the exhaustive searches of the admissible solutions and complicated parameter control[14]. Compared to these techniques, the greedy algorithm based snake model, proposed by Williams[15], shows its simplicity and efficiency by its competitive performance. This greedy algorithm was derived from the dynamic programming method, works directly in the energy-term-space of snakes but does not use exhaustive searches. The greedy algorithm reasonably combines speed, flexibility and simplicity. In this paper, this greedy algorithm based snake model was used to do the edge detection(Fig. 4).

2.4 Feature Extraction

In order to evaluate the feasibility of classification based on an automated segmentation, a basic set of eight features was extracted: fluorescence intensity, circularity, Major-axis-length, Minor-axis-length, entropy, contrast, angular second moment, and inverse difference moment. The latter the four texture features are extracted via co-occurrence matrix. All features are shown in Table 1.

The main procedures of feature vector construction are followed as:

(i) Compute mean μ_X and standard deviation σ_X for each feature.

Table 1 Features extracted from the segmentation images

1.	fluorescence intensity
2.	circularity
3.	major-axis-length
4.	minor-axis-length
5.	entropy
6.	contrast
7.	angular second moment
8.	inverse difference moment

(ii) Construct the vector of all eight features.

(iii) Repeat steps from (i) to (iii) for all segmentation images and calculate all feature vectors of all.

From the comparison of results, we can see that 'greedy snake' can get very perfect result of edge information.

2.5 Classification

The detection part of the system is a classifier using Mahalanobis distance measure to assign each feature vector(i.e. a result image of edge detection) as a class label like apoptosis or non-apoptosis. Formally, the classification consists of learning and classification phases. These phases will be elaborated in the subsequent sections.

Learning procedures:

1. Given L non-apoptosis images, calculate the feature vectors for each image using the feature extraction scheme given above. Consider these vectors as the true feature vectors and name them as $t_i.(1 \le i \le L)$
2. Compute the mean vector m and the covariance matrix K for the feature vectors t_i.

Classification procedures:

1. Given a test image calculate the feature vectors s_i's using the feature extraction scheme given above.
2. Compute the Mahalanobis distance d_i between each feature vector s_i and the mean vector m $d_i = (s_i - m)^T K^{-1}(s_i - m)$
3. Classify a segmentation image Ci for which d_i exceeds a threshold value α as apoptosis, else identify it as non-defective.

$$C_i = \begin{cases} apoptosis & if\ d_i > \alpha \\ non-apoptosis & otherwise \end{cases}$$

The threshold value α is an empirical value from our experiments.

3. Experiment and results

The experiment was implemented with four sets of features from Table 1 to test their discrimination power. One set included all the features, whereas the other three included a subset of these, as shown in Table 2. A subset A includes all features except fluorescence intensity. A subset B includes features 1-4 except texture features. A subset C includes features 1 and 5-8. A subset D includes all features.

Table 2 The subsets of features

Subset	Feature
A	2-8
B	1-4
C	1,5-8
D	1-8

The final experiments result is in Table 3. We can see that using all the features and a subset C is almost identically and show the highest performance. The subsets A and B show a poorer performance, that of the subset B being the worst.

Table 3 Experiment Results

Subset	Results
A	85.2%
B	71.5%
C	93.8%
D	94.1%

To make it clear and compare the results of our new recognition method with the results of traditional FCM method, we choose the best two features of our method, fluorescence intensity and texture (F+T) along with several intrinsic structural parameters of FCM Cell shape, pigment content and protein fluorescence (S+P+P) and again do the recognition process of apoptosis of our samples. The comparison results between the two methods are followed as:

Table 4 Comparison Results

Feature	Results
F+T	93.8%
S+P+P	87.6%

From the comparison results, we can see that our new method has better performance than the traditional FCM method in recognition of apoptosis cells.

4. Conclusion and future work

In this article, an image-based method for the recognition of apoptosis cells has been presented. With the good experiment results, we conclude that in apoptosis recognition fluorescence intensity and texture feature are highly reliable features and can make good recognition results. New features extraction step and more advanced classifier will be considered in our future works.

References

[1] Arends M. J. & Wyllie A. H., Apoptosis: Mechanisms and roles in pathology. Int. Rev. exp. Pathol., Vol.32, pp. 223-254, 1991.

[2] Wyllie, A. H., Apoptosis and the regulation of cell numbers in normal and neoplasic tissues: an overview. Cancer Metast. Rev., Vol.11, No.2, pp. 95-103, 1992.

[3] Y.Ling, W. Priebe, R. Perez-Soler., Apoptosis induced by anthracycline antibiotics in P388 parent and multidrug-resistant cells, Cancer Res., Vol.53, No.8, pp. 1845-1852, 1993.

[4] Yoshida Y., Hypothesis: Apoptosis may be the mechanism responsible for the premature intramedullary cell death in the myelodysplasic syndrome, Vol.7, No.11, pp. 144-146, 1993.

[5] Belloc F., Dumain P., Boisseaum M.R., A flow cytometric method using Hoechst 33342 and propidium iodide for simultaneous cell cycle analysis and apoptosis determination in unfixed cells, Vol. 17, No.1, pp. 59-65, 1994.

[6] Jean-Philippe Vial, Francis Balloc., Study of the apoptosis induced in vitro by antitumoral drugs on leukaemic cells. Leukemia Research, Vol.21, No.2, pp. 163-172, 1997.

[7] Howard M.Shapiro., Practical Flow Cytometry, Third Edition, New York: Alan R. Liss, 1995.

[8] Foracchia, M., Grisan, E., Ruggeri, A., Luminosity and contrast normalization in retinal images. Medical Image Analysis, Vol.9, No.3, pp. 179-190, 2005.

[9] Isitor, G.N., Thorne, R., Comparison between nuclear chromatin patterns of digitalized images of cells of the mammalian testicular and renal tissues: an imaging segmentation study. Computerized medical imaging and graphics : the official journal of the Computerized Medical Imaging Society, Vol.31, No.2, pp. 63-70, 2007.

[10] Gonzalez, R.C., Woods, R.E., Digital Image Processing, second ed. Prentice Hall, 2002.

[11] Otsu, N., A threshold selection method from gray-level histogram. IEEE Transactions on System ManCybernitics, Vol.9, No.1, pp. 62-66, 1979.

[12] Kass M,Witkin A, Terzopoulos D., Snakes: active contour models. Proc. Int'l Conf. on Computer. Vision, pp. 259-68, London, 1987.

[13] M. Kass, A. Witkin, D. Terzopoulos, Snakes: active contour models, Internat. J. Comput, Vol.1, No.4, pp. 321-331.

[14] S.R. Gunn, M.S. Nixon, Arobust snake implementation via a dual active contour, IEEE Trans. Pattern Anal. Mach. Intell. Vol.19, No.1, pp. 63-68.

[15] D.J. Williams, M. Shah, A fast algorithm for active contours and curvature estimation, Computer Vision, Graphics, Image Processing, Vol.55, No.1, 1992.