# Fluorescence Microscopic Image Cell Segmentation

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*Abstract*—**Apoptosis is a process of Programmed Cell Death (PCD) that is a naturally occurring process in the body. The defective apoptosis process will causes extensive variety of diseases, such as cancer, ischemic damage, and etc. Hence, apoptosis is widely applied in disease analysis and disease treatments. How to segment the cells from a Fluorescence Microscope (FM) image is essential in developing an automatic computer-aided system for Apoptosis detection. In this paper, a Fluorescence Microscopic Image Cell Segmenting (FMICS) system is proposed to cut cells off from an image under fluorescence microscope. The experimental results indicate that the FMICS system provides an impressive performance than the compared methods.**

*Index Terms*—**Apoptosis, cell counting, fluorescence microscope, image segmentation.**

## I. Introduction

Apoptosis is the process of Programmed Cell Death (PCD) which is a normal component of the development and health of multicellular organisms [4] [7]. The defective apoptosis process will cause various diseases, i.e. neurodegenerative diseases, cancer, autoimmune diseases, and etc [4] [7].

In general, the physiologist or biologist observes those morphological changes of cell to detect apoptosis through an electron microscope [9]. However, this process is very labor-intensive and infeasible. Therefore, an automatic computer-aided system for reliable detection and monitoring of apoptosis is important.

As on above mentions, in order to efficiently aids the pathologist or biologist. In this paper, a cell segmentation in the fluorescence microscope image for apoptosis, which call "Automatic Fluorescence Microscopic Image Cell Segmentation and Counting" FMICS system, is proposed for segmenting cell and counting the number of the cell in the Fluorescence Microscopic (FM) image. However, in the apoptosis process, the gray-level intensities of the cells may distribute wide range of gray-level intensities; even the intensities of some cells are too weak for human eyes to label them while the cells are losing vitality. Therefore, FMICS system enhances the contrast of cells. And then, FMICS system employs the Adaptable Threshold Decision (ATD) to segment the cells and uses the watershed algorithm based on distance transform to separate the overlapping cells into individual cells. After that, FMICS system counts the segmented cells in a fluorescence microscopic image.

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## II. FMICS System

### A. Preprocessing Stage

#### 1) Color Selector

To remove the effect of the fluorescent dyes in cell segmenting, the FMICS system transforms the color FM image $I_{RGB}$ into a gray-level image $I$. The FMICS system calculates the color histogram of $I_{RGB}$ with *5* bins, which the *5* bins are red, green, blue, cyan, and yellow, respectively. Let ($C_{Ri}$, $C_{Gi}$, $C_{Bi}$) be the RGB color components of the respective color of the *i*-th bin. In $I_{RGB}$, for each pixel $P$ with three color components ($C_R$, $C_G$, $C_B$), $P$ will be thrown into bin $j$ where

$$j = Arg\left(\underset{i=1}{\overset{5}{Min}}\sqrt{(C_{Ri}-C_R)^2+(C_{Gi}-C_G)^2+(C_{Bi}-C_B)^2}\right). \quad (1)$$

Then, the FMICS system projects the $h^{\text{th}}$ pixel $P_h$ with ($C_R$, $C_G$, $C_B$) in $I_{RGB}$ on the line passing through (*0*, *0*, *0*) and ($C_{Rj}$, $C_{Gj}$, $C_{Bj}$), and assigns the value, obtained by projecting ($C_R$, $C_G$, $C_B$) on the line, to the gray-level intensity of the $h^{\text{th}}$ pixel of $I$. After that, the FMICS system changes the gray-level intensity $x$ of each pixel in $I$ into $\frac{x-\min}{\max-\min}\times 255$, where $max$ and $min$ are the maximal and minimal gray-level intensities of all the pixels in $I$. This operation is to stretch the pixel contrast of $I$ from *0* to *255*.

#### 2) Adaptive Filter

Some cells are uneven and there may still exist some holes on the cells in $I$. Therefore, FMICS system performs an adaptive filter to smooth the surfaces and to fill in the holes on the cells. Assumes that $Avg_I$ is the average gray-level intensity of all the pixels in $I$, a windows $W_a$, which consists of $n_a \times n_a$, be the related window of $I(i, j)$, and $Avg_a$ and $Min_a$ are the average and minimal gray-level intensities of all the pixels in $W_a$, respectively. Due to the cells pixels in $I$ are usually brighter than the background pixels, so adaptive filter can be described as following:

$$I'(i,j) = \begin{cases} Avg_a, \text{if } Avg_a \geq Avg_I, \\ Min_a, \text{otherwise.} \end{cases} \quad (2)$$

After that, the adaptive filter normalizes the gray-level intensity of a pixel on $I'$ from *0* to *255*. Consequently, the

$I'$ is transformed into a gray-level image $I_a$.

*B. Cell Segmenting Stage*

*1) Object-Contrast Enhancement*

In the FM image, the intensities of background pixels general are slighter. Hence, the FMICS system selects a threshold $T$ to roughly classify the pixels into background pixels and object pixels. $T$ is described by following:

$$T = Avg_a - (w_a \times \sigma_a) \quad (3)$$

where $Avg_a$ and $\sigma_a$ are the average gray-level intensity and standard deviation of all the pixels in $I_a$, respectively. $w_a$ is a parameter whose range from $0$ to $1$. The $I_B(x,y)$ is given to be:

$$I_B(x,y) = \begin{cases} 1, \text{ If } I_a(x,y) \geq T, \\ 0, \text{otherwise.} \end{cases} \quad (4)$$

After that, FMICS system generates a binary image $I_B$ through $I_a$. In $I_B$, the $1$-bit is the object pixel and the $0$-bit is the background pixel. In this experiment, $w_a$ is set to $0.35$.

The FMICS system gives an index for each isolate object in $I_B$. Let $O_i$ $(x, y)$ be a pixel in $i^{th}$ object $O_i$ located at the coordinates $(x, y)$ on $I_a$. The object-contrast enhancement to enhance contrast of $O_i$ can be formula by following as:

$$O_i'(x,y) = round\left(\left(\frac{O_i(x,y) - Min_{O_i}}{Max_{O_i} - Min_{O_i}}\right)^{\alpha} \times 255\right), \quad (5)$$

$$O_i'(x,y) = 255, \text{if } O_i'(x,y) \geq 255,$$

where $r$ is a parameter to control degree of contrast enhancement. The $Max_{O_i}$ and $Min_{O_i}$ are the maximal and minimal gray-level intensities of all pixels in $i^{th}$ object, respectively. After that, the $O_i$ $(x, y)$ is changed into $O_i'(x,y)$.

*2) Cell Segmentation*

Since gray-level distributions of cells in the FM image have a larger variation. Hence, the FMICS system employs the ATD method to determine a suitable threshold for each object to segment the cells. Assumes $T_{Oi}$ is the threshold obtained by the ATD method from the gray-level histogram of $i^{th}$ Object. Then, the FMICS system generates $I_b$ by the following formula (6):

$$I_b(x,y) = \begin{cases} 1, \text{if } O_i'(x,y) >= T_{Oi} \\ 0, \text{otherwise,} \end{cases} \quad (6)$$

where $I_b$ is a binary image and a $0$-bit (resp. $1$-bit) denotes a black pixel (resp. a white pixel). We denote the black pixel as background pixel and white pixel as cell pixel.

As the capturing FM image is affected by experimental devices and environments, it may generate some noises in the FM image. Generally, the areas of noises general are small; hence FMICS system utilizes this property to remove the noises in the FM image. Let $Avg_a$ and $Sd_a$ represent the average and standard deviation of the areas of all the objects in $I_b$, respectively, and $Area_{Oi}$ is the area of the $i^{th}$ object in $I_b$. The FMICS system removes the $O_i$ from $I_b$, only if $Area_{Oi} < Avg_a - 2Sd_a$.

*3) Overlapping Cells Splitting*

In a FM image, some cells may are overlapped, connected, or aggregated, which will make cell segmentation more difficult and cell counting imprecise. Therefore, the FMICS system decompose overlapped, connected, or aggregated cell. Let $d_{ij}$ be the distance in spatial location between $i^{th}$ and $j^{th}$ contour pixel of the $k^{th}$ object $O_k$ in $I_r$. The maximal one of all the $d_{ij}$'s in $O_k$ is defined as the length $D_k$ of $O_k$, and $D_k$ is adopts to determine whether the $O_k$ is an overlapping cell or not. Let $Avg_d$ and $Sd_d$ be the average and standard deviation of the lengths of all the objects in $I_r$, respectively. If $D_k \geqq Avg_d + Sd_d$, the $O_k$ is regarded as an overlapping object, and then, the FMICS system performs the watershed algorithm to split $O_k$.

To segment the overlapping object in the binary image, the distance transform and watershed algorithm usually are combined to split the overlapping object [12]. If two cells are connected together in the binary image $I_r$, usually only one minimum and catchment basin will be formed in the topographic surface. To use watershed to segment overlapping object, distance transform [2] is applied to transform the image to make it more suitable for watershed algorithm.

The distance transform [2] calculates distance of object pixel from the each pixel of the object (cell) to the nearest background pixel. Chen et al. [3] pointed out that the watershed algorithm provides a good segmented result with "Chessboard" distance transform. Hence, the FMICS system adopts the "Chessboard" distance transform to transform the binary image $I_r$ into a gray-level image. Let $P_1(x_1, y_1)$ and $P_2(x_2, y_2)$ are two pixels located on coordinate $(x_1, y_1)$ and $(x_2, y_2)$ in the $I_r$. The distance $d_{Chessboard}$ between two pixels can be calculated by following formula (7):

$$d_{Chessboard}(P_1, P_2) = \max(|x_1 - x_2|, |y_1 - y_2|) \quad (7)$$

## III. Experimental Results

In the experiment, $35$ fluorescence microscopic images are used as the test images. Each fluorescence microscopic image is consisted of $1392 \times 1040$ pixels with $24$-bits and the total number of cells in the test images is nearly $7750$ cells. Fig. 1 demonstrates a part of the test images. In the experiment, the parameters $n_a$, $w_a$, $\alpha$, $r_1$, and $r_2$ are set to $n_a=5$, $w_a=0.35$, $\alpha=0.7$, $r_1=2$, and $r_2=0.35$.

The aim of the experiment is to explore the performance of the FMICS system, Althoff *et al.*'s [1], Tang *et al.*'s [10], Eddins' [5] and Yan *et al.*'s [11] methods in segmenting the cells on an FM image. In this experiment, the FMICS system is randomly selected $10$ FM images including $892$ cells from $35$ FM images to segment the cells out. Misclassification Error (ME) [8], Relative Area Error (RAE)

[8], Modified Hausdorff Distance (MHD) [8], and Relative Distance Error (RDE) [12] are often adopted to evaluate the segmentation errors.

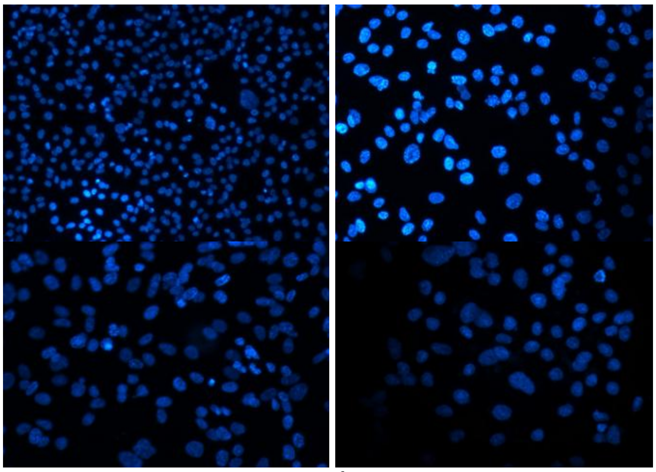
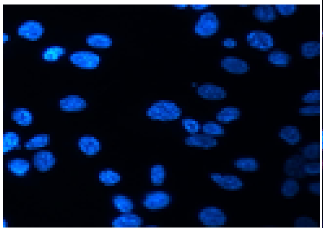
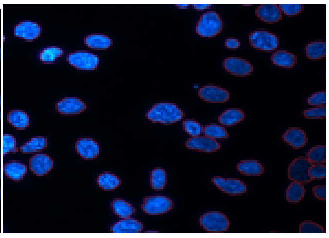


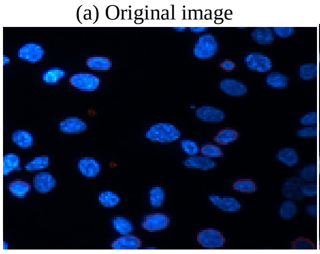
Fig. 1. A part of test images.



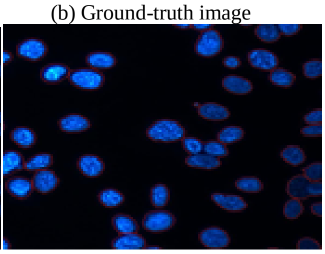
(a) Original image



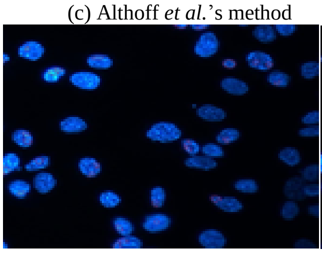
(b) Ground-truth image



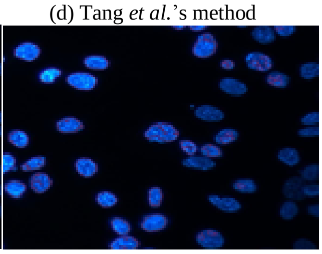
(c) Althoff *et al.*'s method



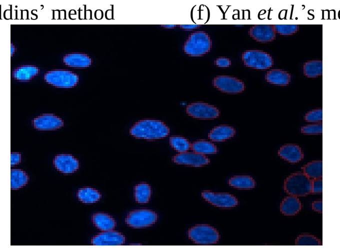
(d) Tang *et al.*'s method



(e) Eddins' method



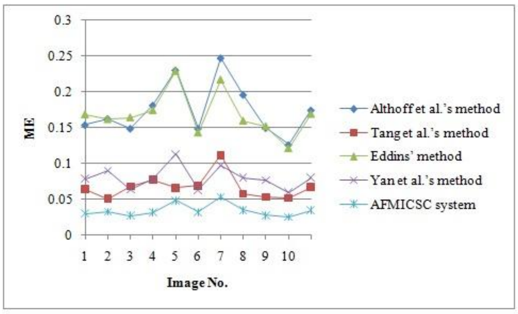
(f) Yan *et al.*'s method



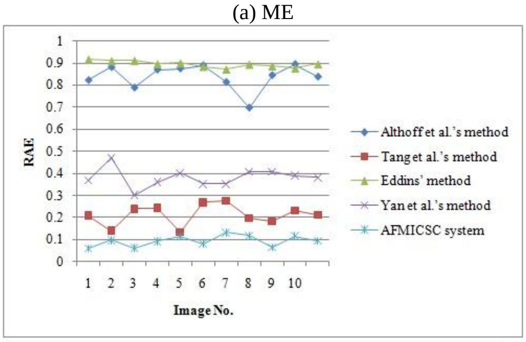
(g) FMICS system

Fig. 2. The ground-truth image and segmented images of a partial FM image obtained by using Althoff et al.'s, Tang et al.'s, Eddins' and Yan et al.'s methods, and FMICS system, respectively.

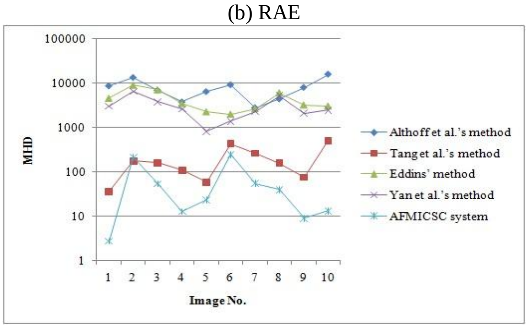
Fig. 2 demonstrates that the ground-true image and segmented images of a partial FM image obtained by FMICS system, Althoff *et al.*'s, Tang *et al.*'s, Eddins' and Yan *et al.*'s methods, respectively. In the Fig. 2, Althoff *et al.*'s and Tang *et al.*'s methods cannot effectively segment the cells; the cells are segmented by using Tang *et al.*'s method, but most segmented cells are more lager than actual sizes of cells in ground-truth image and some overlapped cells cannot separate into distinct cells; Yan *et al.*'s method cannot successful cut off the darker cells; however, the FMICS system can more successfully segment the cells than compared methods. Fig. 3 displays that ME, RAE, MHD, and RDE segmenting errors, respectively. Table 1 shows that the averages of ME, RAE, MHD, and RDE segmenting errors. The experiment results indicate that the FMICS system is better than the other compared methods in segmenting cells.



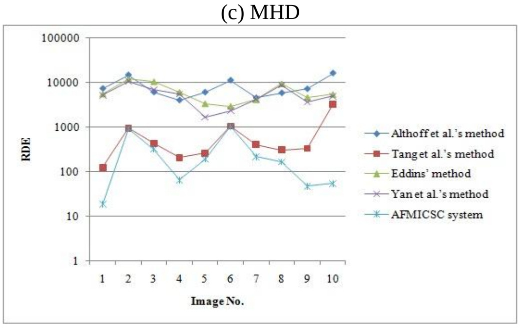

(a) ME




(b) RAE




(c) MHD




(d) RDE

Fig. 3. The ME, RAE, MHD, and RDE segmenting errors.

TABLE I: THE AVERAGES OF ME, RAE, MHD, AND RDE SEGMENTING ERRORS

| | ME | RAE | MHD | RDE |
|---|---|---|---|---|
| Althoff *et al.*'s method | *0.175* | *0.838* | *7946.27* | *8375.75* |
| Tang *et al.*'s method | *0.067* | *0.212* | *197.716* | *735.773* |
| Eddins' method | *0.169* | *0.896* | *4340* | *6439.02* |
| Yan *et al.*'s method | *0.080* | *0.381* | *3048.801* | *5421.21* |
| FMICS system | *0.034* | *0.094* | *66.986* | *304.597* |

## IV. Conclusions

For assisting the physiologist or biologist analyzes properties of cells and counts the number of cell for apoptosis, in this paper, the FMICS system is proposed to automatically segment cell and count the cells in an FM image. The experimental results demonstrate that the FMICS system can effectively segment cells from an FM image than other compared methods. The techniques proposed in this paper also can be applied to segment the objects in other kinds of images.

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