Segmentation and Quantitative Analysis of Normal and Apoptotic Cells from Fluorescence Microscopy Images
Yuncheng Du*, Hector M. Budman*, Thomas A. Duever+

*Department of Chemical Engineering, University of Waterloo, Waterloo, ON, Canada, N2L 3G1
(E-mail: yuncheng du@uwaterloo.ca; hbudman@uwaterloo.ca)
+Department of Chemical Engineering, Ryerson University, Toronto, ON, Canada, M5B 2K3
(E-mail: tom.duever@ryerson.ca)

Abstract: Accurate and fast quantitative analysis of living cells from fluorescence microscopy images is useful for evaluations of experimental outcomes and cells culture protocols. An algorithm is developed in this work to automatically segment and discern apoptotic cells from normal cells. A coarse segmentation algorithm is proposed as a pre-filtering step that combines a range filter with a marching square method. This step provides approximate coordinates of cells’ positions in a two-dimensional matrix used to store cells’ image. With this information, the active contours without edges method is applied to identify cells’ boundaries and subsequently it is possible to extract the mean value of intensity within the cellular regions, the variance of pixels’ intensities in the vicinity of cells’ boundaries and the lengths of the boundaries. These morphological features are then employed as inputs to a support vector machine (SVM) classifier that is trained to distinguish apoptotic from normal viable states of cells. The algorithm is shown to be efficient in terms of computational time, quantitative analysis and differentiation accuracy, as compared to the use of the active contours method without the proposed coarse segmentation step.

Keywords: Cells’ morphology, live-cells imaging, level set function, supervised machine learning

1. INTRODUCTION
Fluorescence microscopy is a well-developed tool to study in vitro cells’ behaviour. However, microscopy experiments can generate a great amount of cells’ images with varying image qualities (Waters, 2009). The manual quantification and analysis of these data is time consuming. Hence, accurate and automatic analysis of cells images such as Chinese Hamster Ovary (CHO) cells can be very useful.

Mammalian cells are prone to apoptosis (programmed cell death), which is a key metabolic event that restricts the growth of cells and decreases the productivity in a bioreactor (Rulter, et al., 2014). The accurate detection of apoptotic cells can help identifying the critical factors that trigger apoptosis. This knowledge may be used for delaying apoptosis and potentially increase the productivity (Taatjes, et al., 2008).

Morphological changes in cells are highly indicative of the occurrence of apoptosis (Henry, et al., 2013). For example, shrinkage and blebbing of the cytoplasmic membrane are found to be significant characteristics of apoptotic cells (see Fig.1), which cause cells to lose normal, smooth and circular shapes. Blebbing during apoptosis is generally associated to swelling of the cell membrane into spherical bubbles. Hence, microscopic observation of morphological changes can be used to discern normal from apoptotic cells. However, cells may exhibit highly variable values of these morphological measures due to the dynamic nature of apoptosis.

This paper presents a new image processing and quantitative analysis method that can automatically differentiate apoptotic from normal cells, while maintaining the computational time at a reasonable level. The proposed method involves three consecutive steps: (i) a coarse segmentation that can be used to identify the number of cells in a given image of cells; (ii) a fine segmentation step to detect the boundaries of cells and to identify particular morphological features related to these boundaries; and (iii) a support vector machine (SVM) based classification model that uses the morphological features identified in the fine segmentation step (step ii) to distinguish apoptotic cells from normal cells.

Our contributions in this work are summarized as follows: (i) a computationally efficient coarse segmentation algorithm that combines a range filter and a marching square method to approximate cells’ locations in an image; (ii) an automated differentiation algorithm to discern apoptotic from normal cells using three morphological features that can be extracted from the results of the fine segmentation algorithm. The method in this work can be easily extended to other studies for real-time monitoring of cells’ cultures and for high throughput screening experiments upon appropriate tuning.

This paper is organized as follows. Section 2 reviews the background on fluorescence imaging and the challenges in analysing cell morphology. The method developed is presented in Section 3. Results and discussion are presented in Section 4 followed by conclusions in Section 5.

2. FLUORESCENCE IMAGING
Fluorescence microscopy has been used to differentiate and quantify apoptotic versus normal cells as well as to determine the viability of cells. This analysis involves two types of fluorescent dyes, i.e., acridine orange (AO) and ethidium bromide (EB), which are mixed in a fixed ratio within the cell suspension which is then analysed by fluorescence microscopy. The AO can penetrate viable and nonviable cells and make cells to appear green while the EB can only diffuse.
into nonviable cells and make them to appear orange (or red). Fig. 1 shows a typical fluorescence photomicrograph of CHO cells stained with AO and EB. As seen, the necrotic cells can be easily distinguished with the pixels intensities, since they appear with an orange-red colour. Thus, in this work, only cells fluorescing green are further studied to differentiate the apoptotic from normal cells.

![Fluorescence photomicrograph of CHO cells with Axiovert 200 (scale bar = 15 μm)](image)

As observed in Fig. 1, apoptotic cells usually exhibit irregular shapes and blurry boundaries, as compared to normal cells. Additionally, the appearance and size of cells belonging to the same class, i.e., normal versus apoptotic, can vary significantly (see Fig. 2). This makes the automatic differentiation of cells status in an image very challenging.

![Examples of cells in different states](image)

In Fig. 2, the cells have different shapes and boundaries. As seen in the first row of cells’ images, normal cells can be characterized by rounded and smooth boundaries. However, the size of normal cells is very different from one another. Apoptotic cells, shown in the second row of images, have very irregular shapes and boundaries. Therefore, a departure from a smooth boundary (blebbing) is a key morphological indicator to discern apoptotic from normal cells. This work builds on the hypothesis that a combination of different indicators such as the average of pixels’ intensities within the cell boundary, a measure of the variability of the pixels’ intensities around the cells’ boundary and the length of this boundary can be used for differentiating normal cells from apoptotic cells.

3. IMAGE PROCESSING METHODOLOGY

3.1 Image segmentation

For images obtained with microscopy, the pixels’ intensities within the cells’ boundaries sometimes are very similar to the intensities measured within the background surrounding the cells. Thus, using strictly an intensity threshold to segment the cells from the background is not effective. Instead, edge-based methods such as the active contour algorithm ignore edges altogether and can handle segmentation more accurately. The central idea behind the active contour algorithms is to iteratively evolve a curve to segment objects from the background which upon convergence provides the boundary. A brief description is given for background.

Assuming a curve $C$, subject to the constraints of a given grayscale image $U_0$ in an open bounded domain $\Omega$ of $\mathbb{R}^2$, which approximates the boundary $\partial$, i.e., $C \approx \partial$. To evolve $C$, the active contours without edges method (Chan & Vese, 2001) seeks a best approximation of $C$ by minimizing an energy function defined as:

$$
\arg \min_{m_1, m_2, C} \mu_1 \cdot \text{Length}(C) + \mu_2 \cdot \text{Area (inside (C))}
$$

$$
+ \lambda_1 \int_{\text{inside}(C)} \left| U_0(x,y) - m_1(C) \right|^2 dxdy
$$

$$
+ \lambda_2 \int_{\text{outside}(C)} \left| U_0(x,y) - m_2(C) \right|^2 dxdy
$$

where $\mu_1, \mu_2, \lambda_1$ and $\lambda_2$ are non-negative tuning parameters, $m_1$ and $m_2$ depend on the evolving curve $C$ and are the mean values of intensities inside $C$ and outside $C$, respectively. The coordinates, defining the domain $\Omega$, are defined by the $x$-axis and $y$-axis. Intensities are available at each point in $x$ and $y$ coordinates. In (1), the first component controls the regularity of $C$ by penalizing its length. The second term penalizes the enclosed area to control the size of the cellular areas. The last two terms penalize the discrepancy between the active curve $C$ and the given image.

The optimization problem (1) can be formulated and solved by a level set method (Osher & Sethian, 1988), where the problem is rewritten in terms of an unknown level set function $Z$. Instead of manipulating $C$, the minimization of (1) is formulated by an equation that progressively evolves the geometric locus of the zero value of the level set function $Z$. Assuming that the unknown level set function $Z$ is smooth, the active contours without edges optimization (1) is written in terms of the level set function $Z$ as:

$$
\arg \min_{m_1, m_2, Z} \mu_1 \cdot \int_{\Omega} \delta(Z(x, y)) \left| \nabla Z(x, y) \right|^2 dxdy
$$

$$
+ \mu_2 \cdot \int_{\Omega} H_\varepsilon(Z(x, y)) dxdy
$$

$$
+ \lambda_1 \int_{\Omega} \left| U_0(x,y) - m_1(Z(x,y)) \right|^2 H_\varepsilon(Z(x,y)) dxdy
$$

$$
+ \lambda_2 \int_{\Omega} \left| U_0(x,y) - m_2(Z(x,y)) \right|^2 (1 - H_\varepsilon(Z(x,y))) dxdy
$$

, where $H_\varepsilon$ is the Heaviside function with respect to the level set function $Z$, and $\delta$ denotes a regularized Dirac $\delta$-function that for the purpose of minimization with respect to $Z$, $H_\varepsilon$ is defined as:

$$
H_\varepsilon = \frac{1}{2} \left( 1 + \frac{2}{\pi} \arctan \left( \frac{Z(x, y)}{\varepsilon} \right) \right)
$$

The minimization of (2) can be solved by updating $m_1$, $m_2$ and $Z$ alternatingly as follows.

For any fixed level set function $Z$, the values of $m_1$ and $m_2$ are the region averages approximated by:

$$
m_1 = \frac{1}{\Omega} \int_{\Omega} U_0(x,y) H_\varepsilon(Z(x,y)) dxdy
$$

$$
m_2 = \frac{1}{\Omega} \int_{\Omega} H_\varepsilon(Z(x,y)) dxdy
$$

$$
m_1 = \frac{1}{\Omega} \int_{\Omega} H_\varepsilon(Z(x,y)) dxdy
$$

$$
m_2 = \frac{1}{\Omega} \int_{\Omega} (1 - H_\varepsilon(Z(x,y))) dxdy
$$

And for fixed $m_1$ and $m_2$ values, a gradient descent equation is formulated for $Z$ with respect to an (artificial iteration) time $t$ as:
As seen in Fig.3 (a), $U_0$ is scanned by a range filter with pixels’ dimensions of $3 \times 3$. The difference of intensities between the maximum and minimum values captured by the range filter around a particular pixel is given in Fig.3 (b). Once the range filtered image $U$ is calculated, a first approximation of the coordinates of cells in $U_0$ can be identified by comparing the intensities in the range filtered image $U$ to a threshold value $\zeta$.

Using this threshold a matrix $U_B$ is generated which elements are binary intensities as follows:

$$U_B = \begin{cases} 1 & \text{if } U(i,j) \geq \zeta \\ 0 & \text{if } U(i,j) < \zeta \end{cases}$$

where $i$ and $j$ represent the coordinates of pixels in the $xy$-plane, $1 \leq i \leq p_1$, and $1 \leq j \leq p_2$.

Subsequently a marching square algorithm is applied to $U_0$ with two objectives: (i) approximate the bounds of cellular regions, (ii) count the number of cells. In principle the main focus of the paper is to distinguish apoptotic from normal cells but it will be shown in the Results’ section that this algorithm is also useful for quickly count cells in the image.

The steps used to approximate contours with the marching square algorithm are schematically shown in Fig.4. Each $2 \times 2$ block of pixels in $U_0$ can be used to construct a contouring grid. The dash line in Fig.4 (a) denotes one contouring grid element generated with the first $2 \times 2$ block of pixels. Fig.4 (b) shows a contouring grid in $U_0$ (dash lines) made of individual contouring grid pieces, which can be used to find a line that all its points have the same intensity value. In our case, since the application of the marching square method follows the thresholding step in (10), this value is 1 (stars in Fig.4 (b)). Since each contouring grid element has 4 corners, there are exactly $2^4$ possible patterns describing portions of the cell contour crossing within each element as shown in Fig.4 (c). By finding a match between the observed lines within each grid element with one of the possible patterns in Fig.4 (c), the contour (dot line) in Fig.4 (b) can be formed.

![Fig. 4 Visual interpretation for generating contours](image-url)
algorithm is only used as a pre-filtering tool to locate the approximate cells’ positions but it must be complemented by the fine segmentation method shown in Section 3.1 to do the final differentiation. It will also be shown in the Results’ section that the RFMS algorithm is very effective and more accurate for the counting of cells, as compared to the level set method applied alone without the RFMS pre-filtering step. Based on the coordinates of contours (the pixels’ location in xy-axis) identified by the RFMS method, the image is divided into sub-images each containing one cell. Then, the segmentation method explained in Section 3.1 is applied to each of the sub-images to perform a finer identification of cells’ boundaries.

3.3 Feature extraction

The differentiation of cells into apoptotic or normal is based on a set of morphological features calculated from the images. Three features are used: (i) the mean value of pixels’ intensities within the cellular regions; (ii) the variance of pixels’ intensities in the vicinity of the boundary and (iii) a measure of the size of the boundary. The choice of these features is justified by a priori knowledge of the phenomena. Apoptotic cells exhibit blebbing due to swell of the cell membrane. This swelling process results in variable fluorescence intensities in the neighbourhood of the cell contour and generally longer contours of apoptotic cells as compared to normal ones thus justifying the properties used here for differentiating cells.

The mean value of pixels intensities of cellular regions is calculated with the level set function as defined in (4), based on the segmentation results obtained in Section 3.1. The calculation of the variance proceeds as per the following steps. (i) Perform the level set algorithm to segment cells from the background. The boundary shown as a solid line in Fig.5 (a) is obtained by connecting the points with a level set function value of Z ≈ 0 in (10). (ii) Set a value N_L that is the number of pixels in the immediate neighbourhood of each point on the boundary to be used for the calculation of the variance (see Fig.5 (b)). (iii) Connect the points neighbouring the boundary defined by N_L to build a fuzzy region around the boundary given by the dash lines in Fig.5 (b). (iv) Calculate the variance of all pixels intensities inside this fuzzy region. (v) Calculate the length of the boundary as the total number of pixels defining the boundary corresponding to pixels with level set function values of zero as calculated by the algorithm in Section 3.1.

3.4 SVM based differentiation

Based on the three features proposed above, a support vector machine (SVM) (Burgers, 1998) classification model is developed to distinguish apoptotic cells from normal cells. SVM was arbitrarily chosen as one possible regression technique among many possible ones such as PLS, etc. A set of training images are selected, and each of the images used for model training is first processed with the RFMS method to approximate the number of cells and determine the sub-images, each containing a cell. Each observed cell is then characterized as either normal or apoptotic cells based on consensus among five different experimentalists and based on the percentage of apoptotic cells at the time the image was taken as determined by available independent flow cytometry data (Mesram, et al., 2011). The morphological features of cells are computed using the method described in Section 3.3. From the images used for model training, the parameters of the SVM model can be optimized with the Matlab® statistics and machine learning toolbox. The trained SVM model is applied to new images that were not used for model training to classify new images into apoptotic or normal. These testing images are also pre-processed with the RFMS method.

The methodology in this work can be summarized as follows: (i) Calibrate the RFMS based coarse segmentation to estimate optimal parameters, i.e., pixels’ dimensions (q_1^2,q_2^2) of the range filter and an intensity threshold ζ in (10). (ii) Generate contouring grids for each training image with marching square method. (iii) Estimate the number of cells and the coordinates of cells. (iv) Construct sub-images with the information obtained in step iii. (v) Characterize manually each of the sub-images as either normal or apoptotic, using experimentalists’ consensus and cytometry. (vi) Perform image segmentation using the level set method and calculate three morphological features for each image classified in step v. (vii) Use the features obtained in step vi as inputs to an SVM classifier trained to discern apoptotic from normal cells.

4. RESULTS AND DISCUSSION

4.1 Coarse segmentation by combining a range filter with a marching square algorithm

One way to assess the advantages of the RFMS algorithm as a pre-filtering tool is by testing its ability to detect the number of cells within an image. To that purpose, the RFMS is applied to a dataset containing 187 cells in 46 images to study the accuracy of the algorithm in terms of cell counts. The range filter with pixels’ dimensions of 3*3 is used to calculate the range map matrix U. The intensity threshold ζ, used to generate the binary matrix U_b, is chosen as 5. Based on U_b, the marching square method is utilized to generate contours, which provide the coordinates and estimated boundaries of cells for a given image U_0. Fig.6 shows the boundaries for two images with different sizes, shapes and physiological states of cells.

Fig. 5 Sketch of the calculation of the second feature

Fig. 6 Summary of quantitative analysis

For comparison purposes, the quantitative analysis of cells is also conducted with the level set method described in Section 3.1 without applying the RFMS step. Following the finding in previous studies (Chan & Vese, 2001; Getreuer, 2012), the
parameters for the level set algorithm are chosen as: \( \mu_1=\mu_2=\lambda_1=\lambda_2=1 \), and the time-step \( \Delta t \) is 0.1. Then, the accuracy in cell counting by the \( RFMS \) with the level set method without the \( RFMS \) step is compared.

When the \( RFMS \) is used, 181 cells are counted correctly and 6 cells are missed. For example, the cell in the circle in Fig.6 (a) was counted as one cell instead of two due to overlapping between two boundaries of neighbouring cells. By contrast, when the level set method without \( RFMS \) is applied to the images only 175 cells are counted correctly and 12 cells are undetected. For example, the region of cells in the circle in Fig.6 (b) was detected as one cell using the \( RFMS \) algorithm. However, it is misclassified as background with the level set method (see Fig.7 (a)). The explanation is that for some cells the importance of the first two terms in (6) which penalize the enclosed area of cells and the regularity of boundaries may be compromised in the level set method as compared to the other two terms in the cost (6).

Further studies are conducted to investigate the cell counting accuracy when the \( RFMS \) is combined with the level set method. For the region in the circle in Fig.6 (b), the level set method is applied to the sub-image generated with the \( RFMS \) method. The sub-image is created by expanding it from both sides by 20 pixels on both \( x \) and \( y \) directions, based on the coordinates obtained with \( RFMS \). The result is shown in Fig.7. As seen in Fig.7 (a), the cells in the circle cannot be detected, if the level set method is applied without the \( RFMS \) to process the original image. However, when the level set method is applied to the sub-image generated with the \( RFMS \) (see Fig.7 (b)), the level set method can successfully segment cells from the background. This confirms that the combination of the \( RFMS \) and level set method is very advantageous, as compared to the level set method applied on its own without the \( RFMS \) pre-filtering step.

**Fig. 7 Comparison of segmentation methods**

4.2 Comparison of image segmentation results

Studies are conducted to investigate the accuracy in terms of segmentation. Fig.8 shows the segmentation results for a few images with the level set method and the \( RFMS \) method.

The analysed cells in Fig.8 have different sizes, shapes and blurry boundaries. The blue lines represent the boundary that is calculated with the level set function, while the red lines are the results approximated with the \( RFMS \) method in this work. As shown in the figure, both methods can successfully segment cells from the background. However, the boundary generated with the \( RFMS \) is less smooth, as compared to the level set method without the \( RFMS \). For example, the first two images of cells (Fig.8 (a) and (b)) have regular shapes and smooth boundaries, but the boundaries calculated with the \( RFMS \) are fuzzy. The differentiation of cells in this work is built upon the hypothesis that low variability is associated with normal cells while higher variability is indicative of apoptotic cells. Thus, the \( RFMS \) must be combined with the level set method since the \( RFMS \) alone generally results in very fuzzy boundaries and provides inaccurate differentiation as shown in the Results’ section.

**Fig. 8 Summary of segmentation results**

4.3 Comparison of computational times needed for segmentation

The computational time is studied for cells’ images that have different sizes with respect to pixels dimensions. Three cases are investigated: \(~800*1100\), \(~550*450\) and \(~150*150\) pixels of cells images. Using the dataset containing a total of 187 cells in 46 images, the average computational times required to only count cells within the images by the level set on its own or the \( RFMS \) on its own are shown in Table 1.

<table>
<thead>
<tr>
<th>Method</th>
<th>Size</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level set</td>
<td>800*1100</td>
<td>601.7</td>
</tr>
<tr>
<td></td>
<td>550*450</td>
<td>20.87</td>
</tr>
<tr>
<td></td>
<td>150*150</td>
<td>2.80</td>
</tr>
<tr>
<td>RFMS</td>
<td>1.18</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>0.31</td>
<td></td>
</tr>
</tbody>
</table>

As seen in Table 1, if the sole objective is to count cells, the computational time of the \( RFMS \) algorithm is significantly lower than the level set based segmentation. Also, as shown in Table 1 the computational times are highly related to the pixels’ dimensions of cells’ images.

The computational time is further investigated by combining the \( RFMS \) with the level set method. Note that the solution of the level set algorithm requires initial conditions (in (6)). We found that an additional benefit of the \( RFMS \) pre-filtering algorithm is that it can provide a good initial guess for the solution of the level set algorithm.

We compared the computational cost of using an initial guess from the \( RFMS \) algorithm versus using a random initial guess for an image containing 3 cells. The computational time is evaluated by the number of iterations that are required to progressively evolve the boundary of cells. It was found that approximately \(~5\) iterations are needed for the level set method to converge to the boundary when the results obtained with \( RFMS \) are chosen as the initial values whereas approximately \(~10\) iterations are required for the randomly chosen initial values. This observation confirms that the ability of the \( RFMS \) to provide an initial guess for the level set algorithm is an additional benefit of using the \( RFMS \) as a pre-filtering step before applying the level set method.
4.4 Feature extraction

The proposed method combines the coarse segmentation step achieved with the RFMS with the fine segmentation achieved with the level set method for the images from dataset. For the training of SVM model, the level set based segmentation is applied to a training set with 100 samples of cells obtained with the RFMS based coarse segmentation. In this training set, 50 images are normal cells and 50 images are apoptotic cells. A feature vector is calculated for each of the training images composed of the 3 proposed morphological features, i.e., the mean value of pixels intensities of cells, the variance of pixels' intensities in the vicinity of the boundary and the complexity of the boundary. A few training feature vectors are shown in Table 2, where the variance is normalized with respect to the mean value of the cellular regions.

<table>
<thead>
<tr>
<th>States</th>
<th>Variance</th>
<th>Complexity</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptotic</td>
<td>9.84</td>
<td>87</td>
<td>74.87</td>
</tr>
<tr>
<td>Apoptotic</td>
<td>9.60</td>
<td>91</td>
<td>81.45</td>
</tr>
<tr>
<td>Apoptotic</td>
<td>6.71</td>
<td>115</td>
<td>64.09</td>
</tr>
<tr>
<td>Normal</td>
<td>5.94</td>
<td>79</td>
<td>74.23</td>
</tr>
<tr>
<td>Normal</td>
<td>8.77</td>
<td>77</td>
<td>84.46</td>
</tr>
<tr>
<td>Normal</td>
<td>3.61</td>
<td>103</td>
<td>91.24</td>
</tr>
</tbody>
</table>

4.5 Differentiation results using a SVM classifier

Using the trained SVM classifier model, 60 images of cells that were not used for the model training are used to test the classification rate, which contain 30 samples of normal cells and 30 samples of apoptotic cells. To evaluate the efficacy of the classification between normal cells and apoptotic cells, a differentiation rate is defined as:

\[ r_{rate} = \frac{d_t}{D_t} \]  

(11)

where \( d_t \) denotes the number of testing images that have been correctly identified and \( D_t \) is the total number of images used for the experiments. To test whether it is necessary to complement the RFMS method with the level set algorithm, two scenarios are studied to discern apoptotic from normal cells, i.e., the combination of the RFMS with level set and the RFMS without the level set method. Table 3 shows the results of differentiation rate \( r_{rate} \).

From Table 3, it can be seen that the combination of the coarse segmentation achieved with the RFMS with the fine segmentation provides high accuracy. The average of \( r_{rate} \) is ~94.5%, and 1 normal cell’s image is misclassified and 2 testing images of apoptotic cells are misidentified. However, 7 of the testing samples of cells (2 normal and 5 apoptotic cells’ images) are misclassified with the RFMS alone that provides a differentiation rate of ~88%.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Normal</th>
<th>Apoptotic</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination</td>
<td>0.96</td>
<td>0.93</td>
<td>0.945</td>
</tr>
<tr>
<td>RFMS</td>
<td>0.93</td>
<td>0.83</td>
<td>0.880</td>
</tr>
</tbody>
</table>

Hence, it is evident that we must combine the RFMS with the level set algorithm, since the standalone application of the level set method without the RFMS cannot provide accurate counting of cells and is time demanding while the use of RFMS without complementation with the level set method results in a differentiation rate that is ~6% lower than the algorithm combining the RFMS and level set methods.

5. CONCLUSIONS

In this work, a methodology has been developed for high throughput screening studies to distinguish apoptotic from normal CHO cells. A simple coarse segmentation algorithm, which combines a range filter and a marching square method (RFMS), is used as a pre-filtering step to provide the approximate positions of cells within each image. Using the information obtained from the RFMS, the level set method is used to achieve the finer segmentation of cells from the background. Based on these segmentation results, three morphological features are computed and used as inputs to train a support vector machine (SVM) classifier, which can accurately classify cells into normal versus apoptotic. The developed algorithm that combines the RFMS with the level set method is shown to be more accurate and significantly faster than the standalone application of the level set method in terms of cell counting or the standalone use of the RFMS in terms of differentiation of apoptotic and normal cells.

ACKNOWLEDGEMENTS

NSERC is acknowledged for the financial support.

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