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Employing Temporal Information for Cell Segmentation Using Max-flow/Min-cut in Phase-Contrast Video Microscopy

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Abstract— **Cell segmentation is a crucial step in many biomedical image analysis applications and it can be considered as an important part of a tracking system. Segmentation in phase-contrast images is a challenging task since in this imaging technique, the background intensity is approximately similar to the cell pixel intensity. In this paper we propose an interactive automatic pixel level segmentation algorithm, that uses temporal information to improve the segmentation result. This algorithm is based on the max-flow/min-cut algorithm and can be solved in polynomial time. This method is not restricted to any specific cell shape and segments cells of various shapes and sizes. The results of the proposed algorithm show that using the temporal information does improve segmentation considerably.**

I. INTRODUCTION

Analysing cell shape and motility is an important process in medical and biomedical studies because most active cellular functions involve change in shape and movements [1]. Manual observation and analysis of cellular images and data sets is a tedious and error prone task. Therefore designing a reliable automatic cell analysis system could considerably ease the burden of this process for biologists. In almost all automated systems, cell detection is a key process, and a reliable cell segmentation system is a crucial module in a cell tracking system.

Among many imaging techniques that are used in microscopic imaging, the two common ones are fluorescent and phase-contrast imaging. In fluorescent imaging, cells are first stained and then tagged by a fluorescence dye. In these images, the cell's nucleus is completely vivid, but the cytoplasm and cell boundaries are not clearly visible [2], [3]. Therefore, these images do not necessarily reflect the shape of the cell [4] and are not appropriate for applications where exact cell boundaries are needed. An alternative imaging technique is phase-contrast, which makes it possible to examine living cells without any staining or fixation prior to imaging [5]. Although phase-contrast images contain both cell nucleus and cytoplasm, poor image quality makes the segmentation task more complex in comparison to fluorescent images.

The most common approach in cell segmentation consists of thresholding, edge detection and morphological operations, which are often applied on the cell nucleus [6], [7], [2]. These methods work well when there is a uniform pixel distribution, which is not the case in phase-contrast images, therefore they are much more effective in fluorescent images. Another widely used technique is active contour [2], [8], [1], which tries to minimize the energy function and evolve a model to fit a cell shape. Although the results of this method are remarkably good, they suffer from being a local optimization [9] and are based on strong cell structure assumptions [10]. An alternative method is to use statistical estimation and employ a Bayesian framework to train classifier(s) on a number of training images. To assign a label (cell/background) to pixels of the input image, it calculates the maximum a posteriori (MAP) probability of each pixel being either a cell or background [11], [12]. In a different approach Bradbury and Wan presented a segmentation algorithm using the normalized cut and spectral clustering [3]. In their approach, after computing the smallest eigenvalues of the Laplacian matrix, the k-means algorithm is used to group the pixels. A major drawback of this method is that spectral clustering does not specify the type (background/cell) of the clustered segments, therefore the authors suggest different approaches such as applying active contours on the bright field image or to use fluorescent image of the cell if available.

All the aforementioned algorithms have the problem of not being modifiable directly by the biologist as they require.

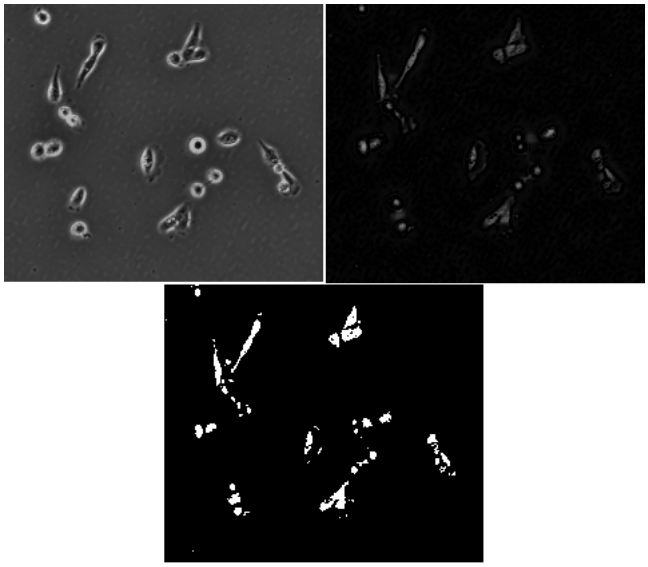

Fig. 1. (Top left) sample image of phase-contrast microscopy, (top right) result of the rolling-ball operation on the same image with cells appearing as white regions, (bottom image) effect of Otsu thresholding on the rolling-balled image.

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Graph cut algorithm has also been used for cell segmentation in florescent images by Lesko et al [13] and Danek et al [14]. Lesko et al's algorithm uses gradient information in the energy function of the graph cut but requires the user to identify a set of points as background/cell. Danek et al suggest the use of the image gradient in the boundary term of graph cut and impose hard constraints on the regional edges after applying thresholding.

In this paper we propose a pixel level cell segmentation algorithm that does not require preprocessing and does not depend on specific cell type or shape. In our approach, we employ the interactive graph cut [15]. In contrast to the original graph cut, which requires manual settings. The proposed method is fully automated while making it possible for the user to correct the results of the algorithm. To make the segmentation even more precise, the temporal information of the cells present in video microscopy is exploited. In what follows we first give a brief description of the general graph cut algorithm and how it can be employed in single frame image segmentation. In section II-B, calculating the graph weights from a training image is described, which is the required step to make the segmentation automatic. Next we extend the single frame segmentation and show how temporal information of cells in video microscopy can be incorporated in the algorithm to improve the segmentation results. An overview of the method appears in Fig.2. We justify our method by providing experimental results.

II. METHODOLOGY

Graph cuts are based on combinatorial optimization and can be computed using the max-flow/min-cut algorithm in polynomial time [16]. The original interactive graph cut algorithm requires the user to specify some pixels (seeds) as background pixels and others as foreground pixels. These seeds are then used to impose hard constraints and the similarity of image pixels to background/foreground pixels is estimated. As we want to minimize user interaction and make the process automatic, we propose to estimate the seeds from a training image. The training image should discriminate cell regions from background regions well. An approximate discriminating image may be computed using the method suggested by Li and Kanade [1], where the rolling-ball morphological operation is applied on the inverted input image. The cells in the resulting image would appear as white shapes; see Fig.1. In order to obtain the binary image from the rolling balled image, Otsu thresholding is applied. The resulting image roughly indicates the cell locations. The next step is to calculate the histogram of the cells (h_c) and the background (h_b) using the binary image as mask. To classify the pixels of a new image, a graph G is created from the image and graph cut is calculated. The calculated histograms (h_c,h_b) are used for estimating the weights of graph edges and they indicate the similarity of each pixel to the background/cell regions as a soft constraint. As contrast and brightness do not vary significantly across the frames in consecutive video microscopy, it is adequate to calculate the histograms only once on the first frame and the same histograms maybe used for the other frames.

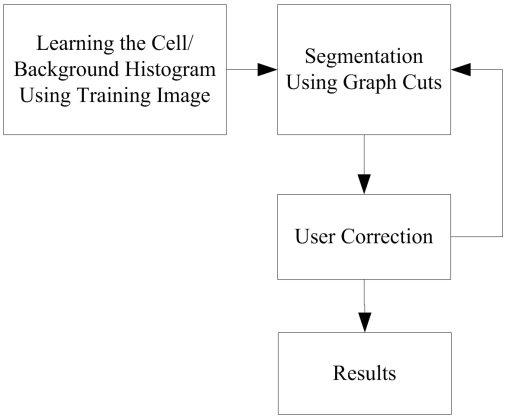

Fig. 2. Methodology

A. Pixel classification using graph-cut

Consider a connected undirected graph $G = \langle V, E \rangle$, in which V is a set of vertices and E is a set of edges with weights ($w \geqslant 0$). The graph has two special terminal nodes, source S associated with the foreground (in our case cells) and sink T which represents the background region. A cut on the graph ($G(C) = \langle V, E \setminus C \rangle$) is defined in such a way that it completely separates the terminal nodes and minimizes the following [15]:

$$\text{minimize}|C| = \sum_{e \in C} w_e \tag{1}$$

In combinatorial optimization problems, often the cost of a cut is defined as the sum of the costs (weights) of the edges in that cut. Graph cut reduces the energy minimization problem to the max-flow/min-cut optimization problem. To use graph cut in image segmentation, each pixel is considered as a node in the graph. For each node two types of edges are created, the edges that connect the node to the terminal nodes (e_t) and the edges that connect each node to its N8 neighbours(e_r). The weights on terminal edges (e_t) specify the regional property of each pixel and show similarity of each pixel to the background and cell region. The weights on the (e_r) edges specify the boundary property of the pixel and avoid discontinuities between pixels with similar intensities. These weight values could be interpreted as cost functions, with the former penalizing the dissimilarity of each pixel to the terminal nodes and the latter keeping the boundary continuous.

B. Weight estimation

Boykov and Jolly [15], use the intensity histogram of the user's defined regions (hard constraints) to estimate the regional penalty (weight on e_t) of pixels elsewhere. However, in automatic segmentation there is no certainty for any region, and no hard constraints can be defined. Therefore we suggest the following energy functions for pixel X :

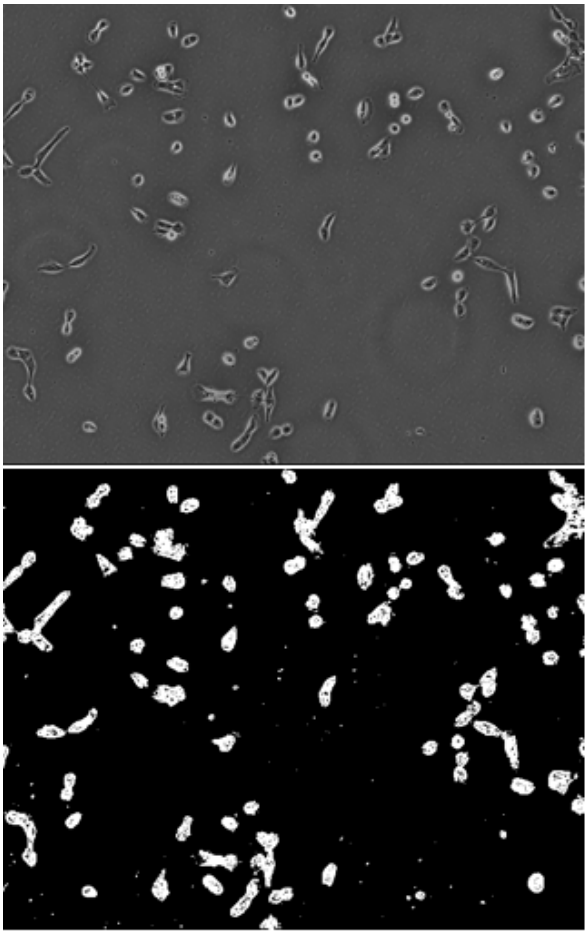

Fig. 3. (Top) is the original image, (bottom) after applying graph cut

$$w_{X,s} = -\ln\left(\frac{h_c(I_X)}{h_c(I_X)+h_b(I_X)}\right)$$
$$w_{X,t} = -\ln\left(\frac{h_b(I_X)}{h_c(I_X)+h_b(I_X)}\right) \quad (2)$$

Recall from the earlier that $h_c(I_X)$ and $h_b(I_X)$ are the normalized values of the learned histograms of the cells and background regions at position X on the training image. To penalize the discontinuities between pixels X and Y with similar intensities, Boykov and Jolly suggest the following weight function :

$$w_{X,Y} = \lambda * \exp\left(-\frac{(I_X - I_Y)^2}{2\sigma^2}\right) \quad (3)$$

Variable λ specifies the relative importance of the discontinuity cost to regional cost. After creating the graph, the algorithm finds the global maximum flow from S to T or the global minimum cut that separates the terminals. This cut divides the image into cell (foreground) and background regions, the result of this process is shown in Fig.3. As mentioned earlier, the graph cut framework makes interactive segmentation possible and gives the user the ability to modify the results. After automatic segmentation, the biologist can

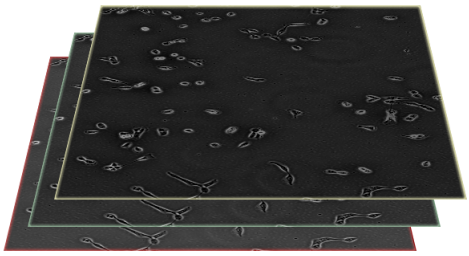

Fig. 4. Stack of three consecutive frames

relabel any misclassified regions. Once the algorithm receives the correction, it imposes hard constraints by setting the new weight value for regional edges. For example if the user relabels a background region to be cell, the algorithm sets, for every pixel X in the region, $w_{X,T} = 0$ and $w_{X,S} = K$ where K is any number larger than all other weights of the computed nearest neighbours and recomputes the cut. This process can be performed efficiently [17]. After finding the pixel classification, the pixels may be grouped together either by computing connected components or the method proposed by Pan et al [10], to extract the cell boundary of each cell.

C. Using temporal information to improve the segmentation

In phase-contrast images, often the cell has the same intensity as the overall background. Therefore when applying graph cut, the cell regions may have discontinuities. Increasing the value of λ does not solve this problem, as it has side effects on other parts of the image, and will continue cell regions into background regions. To overcome this problem, we suggest the use of temporal information which can be incorporated in the application by stacking consecutive frames on top of each other (see Fig.4). The number of frames in the stack may depend on the imaging speed, and the amount of cell movement in consecutive images. Without loss of generality, assume there are 3 frames in the stack. When creating the graph edges for each pixel in frame K, 9 extra edges are created for each of the $K-1$ and the $K+1$ frames. At the end, 26 e_r ($N*9-1$, N= number of frames) edges are created for each node. These edges add extra costs to the dissimilarity of intensity between consecutive frames. This approach can be used in online systems when one frame delay is permitted, or when the only available data is the previous frame. As the graph cut algorithm tries to find the global minima, the outcome of the algorithm will segment all the three frames.

TABLE I

Algorithm	Sensitivity	Specificity
Graph cut	81%	97%
Graph cut with Temporal Information	89%	97%

III. Results

We apply both versions of the proposed algorithm on a cell video provided by Garvan Institute of Medical Research[1]. The video contains 122 frames with frame size 650×515 pixels. The algorithm is implemented in C++ and uses the max-flow implementation of Vladimir Kolmogorov [16]. The values of λ and σ were chosen experimentally to increase Sensitivity and Specificity to 40 and 2 respectively. The algorithm requires 6.110 seconds on Intel 2.67GHz processor with 4GB of RAM. To quantitatively evaluate the algorithm, we compare the outputs of the algorithms with the ground truth images provided by an expert. The sensitivity and specificity is computed as follows :

$$\begin{aligned} \text{Sensitivity} &= \frac{\textit{True Positive}}{\textit{True Positive} + \textit{False Negative}} \\ \text{Specificity} &= \frac{\textit{True Negative}}{\textit{True Negative} + \textit{False Positive}} \end{aligned} \tag{4}$$

True Positive and *True Negative* are the number of the pixels correctly classified, *False Positive* and *False Negative* the number of the pixels incorrectly classified. As shown in Table I, the graph cut algorithm that uses temporal information outperforms the normal graph cut.

IV. Conclusions

This paper presents a single/multi frame pixel level cell segmentation algorithm. The proposed method automatically classifies pixels using the graph cut algorithm by estimating the soft constraints from the training image and it demonstrates how to incorporate cell temporal information to gain better segmentation results. Future work includes an automatic approach to find an optimal number of frames in the segmentation stack, evaluating the algorithm with more images and the use of Ensemble learning to exploit the redundant segmentation results, obtained in consecutive frame segmentation.

V. ACKNOWLEDGMENTS

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