Cell Segmentation in Time-lapse Phase Contrast Data

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Abstract — **The quantitative analysis of cellular migration has found many clinical applications as it can be used in the study of a large spectrum of biological processes such as tumor development and wound healing. These studies are commonly conducted on datasets that consists of a large number of time-lapse images, a fact that rendered the application of human assisted procedures as unfeasible, especially when applied to large datasets. In the development of automatic tracking strategies the problem of robust cell segmentation plays a central role as the segmentation errors have adverse effects on the performance of the overall tracking process. While the phase contrast image data is often characterized by low contrast, changes in the morphology of the cells over time and cell agglomeration, the cell segmentation process is far from a trivial task. In this paper we present a new cell segmentation approach that maximizes the information related to the local contrast between the cells and the background in each image of the dataset. The proposed method has been evaluated on MDCK and HUVEC cellular datasets and experimental results are reported.**

Keywords - cell segmentation, phase contrast images, Otsu thresholding, image enhancement.

I. INTRODUCTION

The study of cell migration is an active area of research since the understanding of the mechanisms that control the cell motility has substantial importance in the development of new therapies. The traditional way to measure the cell motility involves the application of manual procedures, where the molecular scientists initially annotate and then associate the cells in the entire image sequence using basic image processing tools. However, with the advent of new imaging modalities the amount of data that has to be manually evaluated by molecular researchers is constantly increasing. While the availability of large time-lapse image sequences facilitates the precise evaluation of the biological mechanisms associated with the cellular migration, on the other hand it opened a substantial issue when manual procedures are used in the calculation of the motility indicators. Thus, the development of cell tracking solutions that are able to precisely identify and track the cells in large image sequences has become an active area of research.

Cell segmentation is a distinct component of the cell tracking process that entails the robust identification of the cells in all frames of the sequence. Due to the nature of the acquisition process, the cellular data is often characterized by low contrast, intra and inter-frame intensity variations, substantial changes in the cells morphology over the entire sequence and cell agglomeration. All these adverse factors substantially complicate the cell segmentation process and many existing techniques have developed targeted segmentation strategies that were designed for a particular type of cell data. In this regard, a large number of cell segmentation approaches have been proposed and these include methods based on adaptive thresholding [3], watershed [4], grayscale morphological schemes [5] and deformable models [1], [2]. The direct application of thresholding techniques to the identification of the cells in phase contrast data has not been successful due to the large intensity variation within the background and the poor contrast between the cells and background. The accuracy of cell segmentation using active contour and watershed techniques is highly reduced by factors such as the low gradient associated with the cells boundaries, cellular agglomeration and the large migration of the cells in consecutive frames of the time-lapse image sequence.

In this paper we describe the development of a multi-step hybrid cell segmentation scheme where the initial results returned by the Otsu adaptive thresholding are further refined using morphological reconstruction operators that are applied to improve both the detection rate and to reduce the level of false detections. The experiments were conducted using challenging MDCK and HUVEC cellular data.

II. METHOD

The overview of the developed framework is illustrated in Fig. 1. The first step involves the application of the median filter to eliminate the noisy spots and to reduce the variation in the intensity signal within the homogenous regions in the image. The next step implements local image enhancement [6] to improve the contrast between the cell regions and the background, and to facilitate the isolation of the cells in the presence of agglomeration (cell clustering). This is illustrated in Fig. 2(b) where it can be observed the enhancement of the inner cell areas that are defined by the image regions with the lowest intensity signal. The data resulting from the image enhancement process is inverted and the tophat filter is applied to suppress the background intensities. The result of the tophat is shown in Fig. 2(c) and it can be observed that the tophat image data can be roughly approximated with a bimodal distribution. The next step involves the calculation of the threshold using the Otsu scheme [7] which is followed by thresholding the tophat image with hysteresis in the interval $[T-\alpha, T+\beta]$ to merge the sections from the cell areas that were incorrectly removed during the thresholding process (parameters α and β are user defined). The last step is applied to fill the holes in the segmented cells. The result of the entire segmentation process when applied to the image shown in Fig 2(a) is illustrated in Fig 2(d). Fig. 3 depicts the result returned by the proposed algorithm when applied to a HUVEC image.

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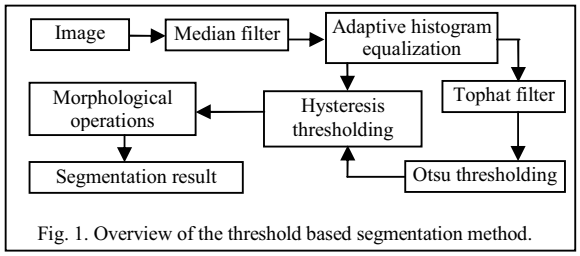

Fig. 1. Overview of the threshold based segmentation method.

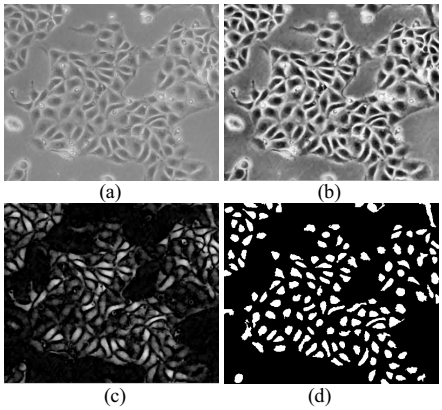

Fig. 2. (a) The original MDCK image. (b) Image resulting from adaptive histogram equalization. (c) Tophat image (inverted). (d) Segmentation results.

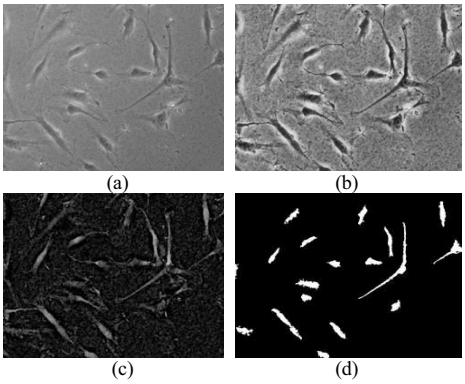

Fig. 3. (a) The original HUVEC image. (b) Image resulting from adaptive histogram equalization. (c) Tophat image. (d) Segmentation results.

III. RESULTS

The proposed method has been evaluated using five image sequences, three MDCK and two HUVEC datasets. The time-lapse phase contrast MDCK datasets are characterized by a large number of cells that are present in each frame of the sequence a property which is in particular useful when assessing the performance of the proposed segmentation technique in the presence of cellular agglomeration. On the other hand the HUVEC cells are large and they are characterized by substantial changes in their morphology when the data is evaluated in consecutive frames of the sequence. As illustrated in Figs. 2 and 3 it can be observed that both MDCK and HUVEC datasets are defined by images characterized by low contrast and high level of noise.

To quantify the accuracy of the proposed cell segmentation method we calculated the following performance metrics: TP – true positive, FP – false positive, FN – false negative, and Accuracy = TP/(TP+FN) and the results are reported in Table 1.

Table 1. Accuracy of the proposed cell segmetation algorithm.

Cell type	No. of frames	Total no. of cells	TP	FP	FN	Accuracy %
MDCK-1	100	16267	15291	253	976	94.00
MDCK-2	100	11525	10910	127	615	94.66
MDCK-3	100	12434	11737	406	697	94.39
HUVEC-1	100	1387	1361	284	26	98.13
HUVEC-2	100	1663	1608	140	55	96.69

IV CONCLUSIONS

In this paper we have introduced a cell segmentation algorithm that combines image enhancement, adaptive thresholding and morphological image reconstruction. We have evaluated the proposed method on five challenging MDCK and HUVEC image sequences and the experimental results indicate that our method is able to identify the cells with an overall accuracy of 95.58%.

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