FAST TRACKING OF FLUORESCENT CELLS BASED ON THE CHAN-VESE MODEL

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ABSTRACT

We present a fast and robust approach to tracking whole fluorescent cells in time-lapse series. The proposed tracking scheme involves two steps. First, coherence-enhancing diffusion filtering is applied on each frame to reduce the amount of noise and enhance flow-like structures. Second, the enhanced cell boundaries are detected by minimizing the Chan-Vese model in a fast level set-like framework. To allow simultaneous tracking of multiple cells over time, the contour evolution has been integrated with a topological prior exploiting the object indication function. Preliminary tracking experiments on 2D time-lapse series of GFP-transfected adipose-derived stem cells demonstrate high accuracy and short execution times.

Index Terms— Cell tracking, fluorescence microscopy, Chan-Vese model, level set framework

1. INTRODUCTION

The ability of cells to exert forces on their environment and alter their shape as they move is essential to various biological processes such as the immune response, embryonic development, or tumorigenesis. Recent technological advances in fluorescent dyes, confocal microscopy, and computing power have given researchers the opportunity to study complex biological processes in great detail. However, the large amount of image data produced in these studies causes that the quantitative analyses of time-lapse experiments rely increasingly on automated or at least semi-automated image processing methods. Namely, there is a high demand for robust and fast approaches to help biologists to reach well-founded conclusions from captured time-lapse series. The crucial task is, in particular, segmenting, tracking, and evaluating movement tracks of cells, subcellular components, and other particles [1].

The methods intended to identify the boundaries of individual cells and track their evolution over time can broadly be classified as either *tracking by detection* or *tracking by model evolution* [2]. The key idea of the former is to detect all cells in each frame of the entire time-lapse series independently and associate the detected cells between successive frames, typically by optimizing a probabilistic objective function. On the contrary, the latter consists in finding cells in the first frame and updating their position and shape frame by frame by considering the result from the previous frame.

Among the latter group of methods, the algorithm based on coupled implicit active contours governed by the modified Chan-Vese model, proposed originally by Dufour *et al.* [3] and improved by Dzyubachyk *et al.* [4], is the most elegant approach for simultaneous tracking of multiple cells currently available. Nevertheless, the representation of each cell by one level set function that is evolved via a numerical solution of a corresponding partial differential equation brings long execution times, even when the narrow band technique is employed.

In this paper, we propose a different tracking by model evolution approach aimed at making the speed as fast as possible, nearly independent of the number of cells to be tracked. In contrast to [3, 4], we detect cells by minimizing the original Chan-Vese model [5] in a fast level set-like framework [6] without solving any partial differential equation, and replace coupling of multiple level set functions with a topological prior exploiting the object indication function [7] to allow simultaneous tracking of multiple cells over time. The tracking experiments on 2D time-lapse series of GFP-transfected adipose-derived stem cells show high accuracy and short execution times. Since the cytoplasm of these cells often contains slender cell extensions, the tracking step is preceded by coherence-enhancing diffusion filtering (CED) [8] that reduces the amount of noise and enhances flow-like structures.

The paper is organized as follows. The Chan-Vese model is reviewed briefly in Section 2. Section 3 describes the proposed tracking scheme. Its experimental evaluation is given in Section 4. Finally, the paper is summarized in Section 5.

2. CHAN-VESE MODEL

This section recalls shortly the background of the Chan-Vese model that constitutes the core of the proposed algorithm.

A functional formulation of image segmentation proposed by Chan and Vese [5] has become popular in the image processing community mainly due to its ability to detect objects not necessarily defined by a gradient. The aim of this model is to partition an input scalar image $u_0 : \Omega \to \mathbb{R}$ defined over a d-dimensional image domain $\Omega \subset \mathbb{R}^d$ into two possibly disconnected regions Ω_1 (foreground) and Ω_2 (background) of

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low intra-region variance and separated by a smooth closed contour C ($\Omega = \Omega_1 \cup \Omega_2 \cup C$). It can be formulated as

$$E_{CV}(C, c_1, c_2) = \mu|C| + \lambda_1 \int_{\Omega_1} (u_0(\mathbf{x}) - c_1)^2 \, d\mathbf{x} + \lambda_2 \int_{\Omega_2} (u_0(\mathbf{x}) - c_2)^2 \, d\mathbf{x} \,, \quad (1)$$

where c_1 and c_2 denote unknowns representing the average intensity level inside Ω_1 and Ω_2, respectively, and μ, λ_1, and λ_2 are positive user-defined weights. The optimal segmentation (C, c_1, c_2) corresponds to a global minimum of (1). The minimization is usually accomplished in the level set, graph cut, or convex optimization framework.

3. PROPOSED TRACKING SCHEME

A description of the proposed tracking scheme is introduced in this section. Since the cytoplasm of analyzed cells often consists of several regions connected by thin fiber-like structures (Fig. 1a), the proposed tracking scheme involves two steps. First, a coherence-enhancing diffusion filter [8] is applied on each frame to reduce the amount of noise and enhance flow-like structures. Second, the cell boundaries are detected by minimizing the Chan-Vese model in the fast level set-like framework [6]. The initial models established for the cells detected in the first frame are evolved in time to fit the particular cells in the subsequent frames. To handle dividing and touching cells over time, a topological prior exploiting the object indication function [7] is applied. It provides a simple mechanism for keeping contours of previously isolated cells separated even if the cells touch in the subsequent frames. Simultaneously, it allows each contour to adapt freely to topological changes of the corresponding cell. In the rest of this section, we describe further individual steps of the proposed tracking scheme and explain how to detect cells in the first frame and deal with overlapping and entering cells.

3.1. Coherence-Enhancing Diffusion Filtering

Coherence-enhancing diffusion filtering [8] is a mathematically well-founded method based on the solution of a nonlinear anisotropic diffusion equation. This method, in contrast to common median and Gaussian filters, can enhance flow-like structures while reducing the amount of noise, thus allowing the detection of slender, elongated cell extensions (Fig. 1). For preprocessing purposes, only a few iterations of the semi-implicit scheme stabilized by an additive operator splitting [8] are needed. We performed two iterations with fixed time step $\Delta t = 1.25$, noise scale $\sigma = 1$, and integration scale $\rho = 3$ in all experiments. The choice of σ and ρ reflects the width of the thin fiber-like structures to be preserved. The relatively low number of iterations stems from the fact that dominating photon shot noise is primarily reduced during the first few iterations of the diffusion process.

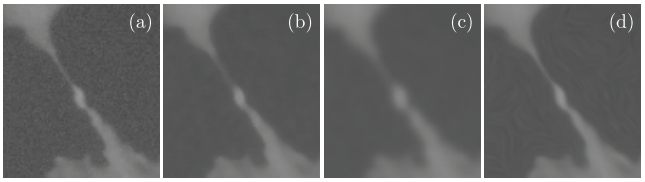


Fig. 1. Filtering effect on a thin fiber-like structure. (a) Original image data. (b) Median filter for a square window ($r = 2$). (c) Gaussian filter for $\sigma = 2$. (d) Coherence-enhancing diffusion filter for $\sigma = 1$, $\rho = 3$, and $t = 2.5$.

3.2. Segmentation of the First Frame

Since the number of cells in the first frame is not known in advance, the cell boundaries are detected using the original topology-flexible Chan-Vese model [5]. To avoid any user interaction, the fast level set-like framework [6] is initialized using a fully automated weighted 2-means clustering [9] that minimizes (1) without the regularization term and provides a suitable initial contour, given λ_1 and λ_2. Once a steady state is reached, small components enclosing foreign particles such as dust are discarded from the final binary mask. The remaining components are assigned different object indicators and established as the cells to be tracked. The criterion for determining whether a component is discarded or not compares its size in pixels with a user-defined threshold s_r that reflects *a priori* knowledge of the size of tracked cells.

3.3. Overlapping Cells

By *overlapping cells*, we mean the situation when the same spatial position is occupied by different cells in two successive frames. In these situations, the initial seed of one cell might expand within the overlapped cell, thus yielding undesired results (Fig. 2b). Let w_o denote the diameter of the overlap. Clearly, the overlap size is bounded by w_o^2. If w_o is only a few pixels, the initial seed of one cell can be forced out of the overlapped cell by strengthening the regularization term in (1). Unfortunately, stronger regularization also breaks thin fiber-like structures of the cell cytoplasm. We propose, therefore, an alternative strategy that involves expanding the initial seed in the overlapped cell and changing the object indicators upon convergence. Given a component m_1 of the object indicator l_1 and of the size smaller than w_o^2, which touches a component m_2 of a different object indicator l_2 (Fig. 2b), its object indicator is changed from l_1 to l_2 (Fig. 2c).

3.4. Capturing Entering Cells

New cells entering the field of view must be paid special attention. They have not been detected in the previous frames and are, therefore, considered as a part of the image background. This can affect significantly the background statistics and lead to incorrect segmentation of a particular frame.

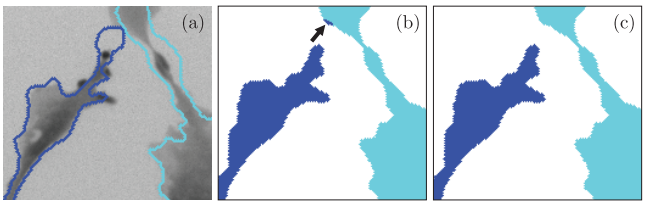


Fig. 2. Tracking of overlapping cells. (a) Inverted original image data overlaid with the final contours from the previous frame. (b, c) Segmentation results for $w_o = 0$ and $w_o = 10$.

To overcome this difficulty, Dzyubachyk *et al.* [4] proposed to segment the current frame without taking entering cells into consideration. Then, create new level set functions for candidates of entering cells and evolve them from the current positions. Since the contours of all existing cells have already converged, this process takes relatively short time to complete. However, creating new level set functions for the canditates can change the background statistics. Thus, the already converged contours of existing cells could attain different positions if their evolution would have also resumed. To assure that we start segmenting the current frame using the appropriate background statistics, we propose instead to add the candidates directly to the final result from the previous frame, assigning to them object indicators different from the existing ones. The candidates are chosen from a coarse segmentation obtained using the weighted 2-means clustering [9], being border components disjoint with the existing cells.

4. EXPERIMENTAL RESULTS

The experimental evaluation of our algorithm, conducted on a common workstation (Intel Core i7-880 3.07 GHz, 3 GB RAM, Windows XP Professional), is given in this section.

The performance was tested on three 2D time-lapse series of GFP-transfected adipose-derived stem cells acquired using a Zeiss Cell Observer Spinning Disk confocal microscope equipped with a dry Plan-Apo 20×/0.85 objective lens. Each time-lapse series had 19 frames of the size 512×478 pixels (332.8×310.7 µm) taken every 5 minutes. The acquired 16-bit image data, compensated for uneven illumination using a flat-field correction procedure, contained from one up to five cells, 10 cells in total, having a bright nucleus within a lower intensity, heterogeneous cytoplasm of a complex shape.

Based on the average cell size, the parameter s_r was fixed at 400 pixels. It corresponded roughly to 20 % of the minimal average size of a complete cell (i.e., a cell not touching the image border in the first frame) and allowed detecting and tracking cells that appear only partly in the field of view (Fig. 4). The occurrence of overlapping cells was very sporadic in the analyzed time-lapse series. When some overlapping cells appeared, their overlap was only in the order of a few pixels in diameter. Therefore, the parameter w_o was fixed at 10 pixels.

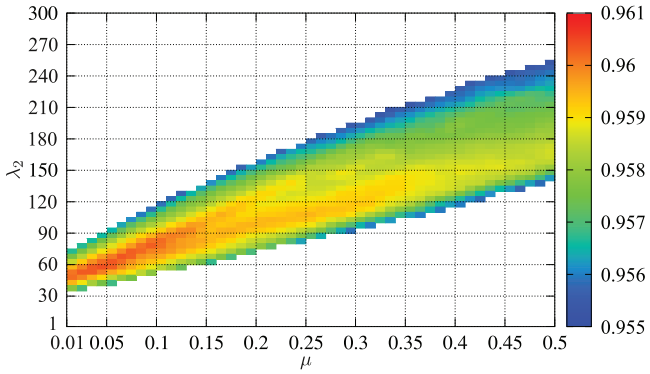


Fig. 3. Thresholded accuracy map of the Jaccard coefficient at the level $JC_{\text{avg}} = 0.955$ for the proposed tracking scheme.

In order to find the optimal values of μ, λ_1, and λ_2, for which the proposed tracking scheme achieves the highest accuracy with respect to a manual segmentation done by an expert, 3050 different configurations: $\mu = 0.01, 0.02, \ldots, 0.50$, $\lambda_1 = 1$, and $\lambda_2 = 1, 5, 10, \ldots, 300$, were examined. The Jaccard coefficient JC given, for a ground truth X and segmentation result Y, as $JC = |X \cap Y|/|X \cup Y|$ was considered as the accuracy measure. It was evaluated separately for each of 5 complete cells and averaged over all time-lapse series. The thresholded accuracy map at the level $JC_{\text{avg}} = 0.9550$ is depicted in Fig. 3. The highest accuracy, $JC_{\text{avg}} = 0.9606$, was achieved for the configuration ($\mu = 0.07, \lambda_1 = 1, \lambda_2 = 70$). An example of the tracking results is shown in Fig. 4.

The second experiment was aimed at the comparison of the proposed tracking scheme (MAS) with the algorithm by Dzyubachyk *et al.* (DZY) [4]. Its parameters were optimized to achieve the best possible results: weight of the regularization term $\alpha = 1.5$, number of iterations $n_i = 10$ after which the foreground and background statistics are recomputed, narrow band width $k = 3$, and time step $\Delta t = 0.1$. To obtain comparable results, DZY was applied on the preprocessed image data after CED and precompiled Matlab interfaces of both algorithms were considered. The average accuracy JC_{avg} and execution time needed for analyzing each of the tested time-lapse series are listed in Table 1. In comparison to DZY, MAS achieved about 3 % higher accuracy and was about one order of magnitude faster. Furthermore, its execution time was almost the same for all three tested time-lapse series with the increasing number of tracked cells (one, four, and five cells). However, MAS requires a manual separation of cells clustered in the first frame in order to track each of them correctly over time. This limitation will be addressed in future work.

5. CONCLUDING REMARKS

In this paper, we have proposed a fast and robust approach to tracking fluorescent cells in time-lapse series. The proposed

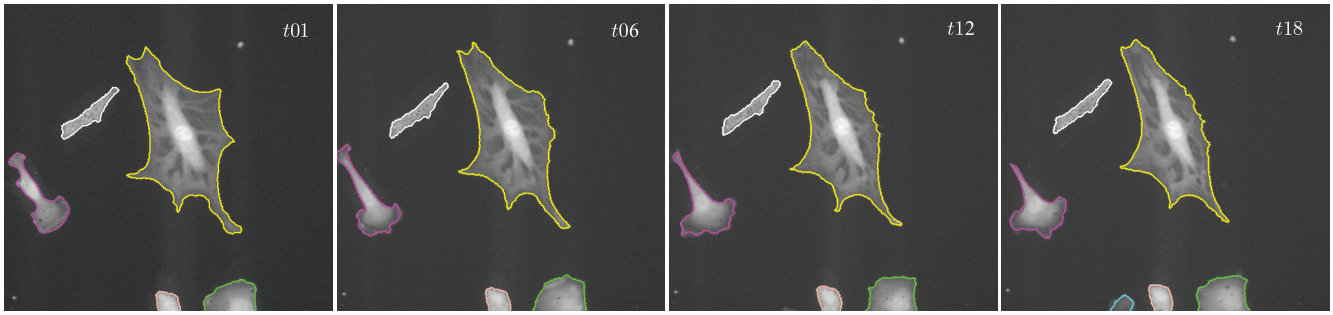


Fig. 4. Results of the proposed tracking scheme for the frames 1, 6, 12, and 18. Original image data overlaid with the final contours. Different colours correspond to different object indicators. New entering cell captured in the frame 18.

Table 1. Average accuracy JC_{avg} and execution time (CED + tracking step) of the compared methods for three tested series with the increasing number of cells (one, four, and five cells).

		Execution Time (s)		
Method	JC_{avg}	Series 1	Series 2	Series 3
DZY	0.9331	29.29	34.56	38.04
MAS	0.9606	3.13	3.20	3.17

tracking scheme combines coherence-enhancing diffusion filtering with the fast level set-like framework that minimizes the Chan-Vese model. It allows simultaneous tracking of multiple cells over time by applying a topological prior based on the object indication function. The preliminary experiments on 2D time-lapse series of GFP-transfected adipose-derived stem cells showed its high accuracy and short execution time.

Although we have focused only on 2D time-lapse series in this paper, the proposed tracking scheme can directly be used in 3D as well. Furthermore, its application is not limited only to tracking whole fluorescent cells. The topological flexibility of the object indication function is also appropriate to handle mitotic cell division events, thus allowing tracking of cell nuclei. We are currently undertaking a thorough experimental evaluation of the proposed tracking scheme on 3D time-lapse series of various cell types. The preliminary experiments on these 3D time-lapse datasets, having completely different intensity distributions, revealed that only the parameter λ_2 needs to be optimized. The other two parameters of the Chan-Vese model can be fixed. Furthermore, given fixed μ and λ_1, the range of λ_2 for which the proposed tracking scheme achieves almost the best possible accuracy is relatively wide, as illustrated also in Fig. 3.

6. ACKNOWLEDGMENT

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7. REFERENCES

[1] E. Meijering, I. Smal, and G. Danuser, "Tracking in molecular bioimaging," *IEEE Signal Proc Mag*, vol. 23, no. 3, pp. 46–53, 2006.

[2] C. Zimmer, B. Zhang, A. Dufour, A. Thébaud, S. Berlemont, V. Meas-Yedid, and J.-C. Olivo-Marin, "On the digital trail of mobile cells," *IEEE Signal Proc Mag*, vol. 23, no. 3, pp. 54–62, 2006.

[3] A. Dufour, V. Shinin, S. Tajbakhsh, N. Guillén-Aghion, J.-C. Olivo-Marin, and C. Zimmer, "Segmenting and tracking fluorescent cells in dynamic 3-D microscopy with coupled active surfaces," *IEEE Trans Image Proc*, vol. 14, no. 9, pp. 1396–1410, 2005.

[4] O. Dzyubachyk, W. A. van Cappellen, J. Essers, W. J. Niessen, and E. Meijering, "Advanced level-set-based cell tracking in time-lapse fluorescence microscopy," *IEEE Trans Med Imag*, vol. 29, no. 3, pp. 852–867, 2010.

[5] T. F. Chan and L. A. Vese, "Active contours without edges," *IEEE Trans Image Proc*, vol. 10, no. 2, pp. 266–277, 2001.

[6] M. Maška, P. Matula, O. Daněk, and M. Kozubek, "A fast level set-like algorithm for region-based active contours," in *Int Symp Visual Computing*, 2010, pp. 387–396.

[7] M. Maška, P. Matula, and M. Kozubek, "Simultaneous tracking of multiple objects using fast level set-like algorithm," in *Sixth Doctoral Workshop Math and Eng Methods in Comp Science (Selected Papers)*, 2011, pp. 69–76.

[8] J. Weickert, "Coherence-enhancing diffusion filtering," *Int Jour Comp Vis*, vol. 31, no. 2/3, pp. 111–127, 1999.

[9] F. Gibou and R. Fedkiw, "A fast hybrid k-means level set algorithm for segmentation," in *Annual Hawaii Int Conf Statistics and Mathematics*, 2005, pp. 281–291.