Joint Tracking of Cell Morphology and Motion

Jierong Cheng^{1,2,*}, Esther G.L. Koh^{3,**}, Sohail Ahmed³,
and Jagath C. Rajapakse^{1,2,4}

¹ BioInformatics Research Center and School of Computer Engineering,
Nanyang Technological University, Singapore
jrcheng@ntu.edu.sg

² Singapore-MIT Alliance, Singapore

³ Institute of Medical Biology, Singapore

⁴ Department of Biological Engineering,
Massachusetts Institute of Technology, USA

Abstract. A new method is proposed for joint tracking of cell morphology and motion from 3D temporal cellular images. We adopt the framework of region-based active contours for segmentation, which is able to cope with objects having blurred boundaries. Motion estimation is performed by optical flow to increase the robustness and accuracy. Cell morphology and motion are modelled via a unified energy formulation and estimated iteratively searching for the minimum energy configuration. Experiments are carried out on synthetic and real cellular images to demonstrate the performance of the method.

Keywords: Cell segmentation, tracking, motion estimation, optical flow, active contours, level sets.

1 Introduction

Three-dimensional (3D) molecular imaging techniques are now capable of generating large time-lapsed image datasets rapidly. Manual segmentation of cells in these images is almost impossible. Automatic segmentation and tracking methods are therefore necessary to enable quantitative understanding and analysis of cellular images.

In recent years, many image analysis approaches have been adopted for cell segmentation from microscopic images. Parametric active contours, or snakes, are widely applied in boundary detection and motion tracking [1],[2]. Geometric active contours based on level sets are becoming increasingly popular because they are not constrained by the topology and thus are capable of detecting an arbitrary number of cells from an arbitrary initial front. In the model of active contours without edges [3], the image energy terms are computed using intensity variances inside and outside the contour. This region-based approach provides strong robustness to noise and allows segmentation of cells with blurred edges.

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* Corresponding author.

** Esther G.L. Koh is now with the Singapore Immunology Network, Singapore.

The approach has been used for segmenting and tracking cells in 2D images [4], [5], [6] and 3D images [7].

Much work has been devoted to the data analysis on time-lapsed cellular images. Existing cell tracking methods can be roughly divided into frame-to-frame linking and mathematical shape modelling methods. Promising methods for cell tracking include hidden Markov modelling [8], dynamic programming optimization [9], Kalman filter [10], and optical flow [11]. In an overview of cell motility analysis and particle tracking methods [12], it is concluded that regional oriented models are far more robust than purely edge oriented models.

Optical flow is a convenient and useful representation of image motion. Discontinuities in the optical flow can help in segmenting images into regions of different motions. By direct coupling of a motion field and a segmentation map, a dense optical flow field which is boundary-preserving can be computed, without relying on specific constraints of motion boundary. Joint motion estimation and segmentation by combining optical flow with active contours has been applied on photographic images in [13], [14], [15], and [16]. The energy functional proposed by Feghali [14] embeds the constraints of both the motion estimation and segmentation. The spatial-temporal surface evolution is entirely driven by the magnitude of the motion field. However, 3D temporal images are often of low resolution and the cell boundaries are blurred and noisy. The existing gradient-based methods have difficulty in dealing with these images. Based on the method proposed by Feghali, we introduce regional force in the curve evolution which proved efficient in segmenting cells with blurred edges.

This work is motivated by the study of neuronal cell growth. In order to obtain basic understanding of cell structure development, cell morphology, and motion need to be tracked accurately from 3D temporal cellular images. It is of great interest to know how neuronal stem cells grow into mature neurons. The quantitative measurements are important as cells are allowed to grow. We propose an automatic technique to quantify the growth of cell by simultaneously tracking cell morphology and motion. The proposed method is more robust and accurate for 3D cell segmentation than the existing methods, by combining optical flow and region-based active contours. The paper is organized as follows. In Section 2, a short overview of active contours and optical flow is introduced and our combining model is described. Experimental results are shown in Section 3 and the conclusion is given in Section 4.

2 Method

2.1 Region-Based Active Contours

Active contours compute segmentations of a given image by evolving contours in the direction of the negative gradient of image energy. Traditional active contours make use of image gradient to localize object boundaries. For cell segmentation from 3D microscopic images, we choose to adopt the model of active contours without edges [3]. In this region-based model, image energy is computed from

surface integrals over the entire image. Therefore, region-based models are robust to noise and allow segmentation of objects with blurred edges. The active contours are implicitly represented by a single level set function and the topology of objects changes automatically as the level set function evolves. This enables automatic detection of an arbitrary number of cells from an arbitrary initial front.

Let $I(\omega, t)$ be the image intensity at location $\omega = (x, y, z) \in \Omega$ at time t , $\Omega \subset \mathbb{R}^3$ being the 3D image domain. $\phi(\omega, t)$ is a level set function defined on Ω , whose zero-level set $\{\omega \in \Omega | \phi(\omega, t) = 0, \forall t\}$ defines the segmentation such that the value of $\phi > 0$ inside the segmented objects and $\phi < 0$ outside. The energy function of the active contour is based on a reduced form of the Mumford-Shah function for image segmentation (we drop ω for notational simplicity):

$$E(\phi, I) = \alpha \int_{\Omega} \delta(\phi) |\nabla \phi| d\omega + \lambda_I \int_{\Omega} \mathcal{H}(\phi) (I - \mu_I)^2 d\omega + \lambda_O \int_{\Omega} (1 - \mathcal{H}(\phi)) (I - \mu_O)^2 d\omega \quad (1)$$

where $d\omega$ is the elementary volume; μ_I and μ_O are respectively the mean intensity of pixels inside and outside the zero level-set; $\mathcal{H}$ and δ are the Heaviside and Dirac functions; λ_I , λ_O , and α are positive constants.

The minimization of image energy is accomplished by letting the level set function evolve as a function of an abstract time t , starting from an initialization $\phi(\omega, t = 0)$ according to:

$$\frac{\partial \phi}{\partial t} = \left(\alpha \nabla \cdot \frac{\nabla \phi}{|\nabla \phi|} - \lambda_I (I - \mu_I)^2 + \lambda_O (I - \mu_O)^2 \right) \delta(\phi). \quad (2)$$

Here $\nabla \cdot \frac{\nabla \phi}{|\nabla \phi|}$ is the (mean) curvature of the level set, generating a regulating force which smoothes the contours. The other two forces on the righthand side move the contour toward the actual boundary of objects.

2.2 Optical Flow Field

Let $u(\omega, t)$ and $v(\omega, t)$ be the x and y velocity component of the apparent motion of brightness patterns of the image, respectively. The classical regularization of the optical flow field used by Horn and Schunk [17] has a quadratic functional:

$$\min_{(u,v)} \int_{\Omega} (u_x^2 + u_y^2 + v_x^2 + v_y^2) d\omega \quad (3)$$

subject to the optical flow constraint

$$I_x u + I_y v + I_t = 0. \quad (4)$$

where $u_x = \frac{\partial u}{\partial x}$, $u_y = \frac{\partial u}{\partial y}$, $v_x = \frac{\partial v}{\partial x}$, and $v_y = \frac{\partial v}{\partial y}$ are optical flow gradients; I_x , I_y , and I_t are the intensity gradients in the x , y , and the temporal directions,

respectively. Horn and Schunk's smoothness constraint seeks to improve some measure of consistency on the flow vectors that are close to each other.

The energy function combining the smoothing error and image motion error is

$$E(I, u, v) = \int_{\Omega} [\beta(u_x^2 + u_y^2 + v_x^2 + v_y^2) + (I_x u + I_y v + I_t)^2] d\omega \quad (5)$$

2.3 Joint Tracking of Cell Morphology and Motion

We propose the energy formulation for joint tracking of cell morphology and motion.

$$\begin{aligned} E(\phi, I, u, v) = & \alpha \int_{\Omega} \delta(\phi) |\nabla \phi| d\omega + \lambda_I \int_{\Omega} \mathcal{H}(\phi) (I - \mu_I)^2 d\omega \\ & + \lambda_O \int_{\Omega} (1 - \mathcal{H}(\phi)) (I - \mu_O)^2 d\omega \\ & + \int_{\Omega} [\beta(u_x^2 + u_y^2 + v_x^2 + v_y^2) + (I_x u + I_y v + I_t)^2] d\omega \\ & + \gamma_I \int_{\Omega} \mathcal{H}(\phi) \frac{1}{1 + u^2 + v^2} d\omega + \gamma_O \int_{\Omega} (1 - \mathcal{H}(\phi)) (u^2 + v^2) d\omega \end{aligned} \quad (6)$$

where γ_I and γ_O are positive constants. The last two terms of the right hand side of (6) correspond to motion segmentation constraints: large motion field is expected in the spatiotemporal region that encloses moving objects; small motion field is expected in the spatiotemporal region that encloses background.

The evolution equations for (ϕ, u, v) is derived by applying Euler-Lagrange equations on (6). The level set evolution is given by the following descent equation

$$\begin{aligned} \frac{\partial \phi}{\partial t} = & \left(\alpha \nabla \cdot \frac{\nabla \phi}{|\nabla \phi|} - \lambda_I (I - \mu_I)^2 + \lambda_O (I - \mu_O)^2 \right. \\ & \left. - \gamma_I \frac{1}{1 + u^2 + v^2} + \gamma_O (u^2 + v^2) \right) \delta(\phi). \end{aligned} \quad (7)$$

The minimization of the energy function with respect to the motion field (u, v) yields a set of two descent equations that can be solved iteratively by

$$\begin{aligned} u = & \frac{\beta(I_x^2 + \beta + \varepsilon) \bar{u} - \beta I_x I_y \bar{v} - (\beta + \varepsilon) I_x I_t}{(\beta + \varepsilon)^2 + (\beta + \varepsilon)(I_x^2 + I_y^2)} \\ v = & \frac{\beta(I_y^2 + \beta + \varepsilon) \bar{v} - \beta I_x I_y \bar{u} - (\beta + \varepsilon) I_y I_t}{(\beta + \varepsilon)^2 + (\beta + \varepsilon)(I_x^2 + I_y^2)} \end{aligned} \quad (8)$$

where

$$\varepsilon = -\frac{2\gamma_I}{1 + \bar{u}^2 + \bar{v}^2} \mathcal{H}(\phi) + 2\gamma_O (1 - \mathcal{H}(\phi)) \quad (9)$$

and

$$\bar{u}(\omega, t) = \frac{\sum_{(\omega_i, t) \in N_{\phi}(\omega, t)} u(\omega_i, t)}{|N_{\phi}(\omega, t)|} \quad (10)$$

with $N_\phi(\omega, t) = \{w' \in \mathcal{N}(\omega, t) | \text{sign}[\phi(w, t)] = \text{sign}[\phi(w', t)]\}$, $\mathcal{N}(\omega, t)$ being the neighborhood of (ω, t) . In other words, $\bar{u}(\omega, t)$ is the average take only in the region of the moving objects or that of the static background. Similarly, the expression for $\bar{v}$ is defined.

The proposed algorithm is summarized in Algorithm 1.

Algorithm 1. Joint tracking of cell morphology and motion

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begin
 $t = 0$ 
 $\phi(\omega, t)$  as a small circle at the center of the image
 $u(\omega, t) = v(\omega, t) = 0, \forall \omega \in \Omega$ 
repeat
  for all  $\omega \in \Omega$  do
    Update  $u(\omega, t)$  and  $v(\omega, t)$  according to (8)
    Update  $\phi(\omega, t)$  according to (7)
  end for
   $t = t + 1$ 
until convergence
end

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3 Experimental Results

Experiment is performed on synthetic images as well as on real 3D temporal cellular images. Our results are compared with the method proposed by Feghali [14].

3.1 Synthetic Data

In the original synthetic image shown in Fig. 1(a), the intensity on the square-shaped object is set to be the Euclidean distance to the object boundary. This is aimed to simulate real microscopic images where the intensity contrast at the cell boundary is relatively low. The image at the next time point is generated by moving the same object one pixel to the bottom-right. An optical flow field (Fig. 1(d)) is estimated from two noise-free images. The noise-free image and the corresponding optical flow field are regarded as ground-truth for object segmentation and motion estimation. The images are added with Gaussian noise with mean of 0 and standard deviation of 0.02. The parameter values were taken heuristically for our experiments on synthetic image are: $\alpha = 0$, $\lambda_I = 1$, $\lambda_O = 1$, $\beta = 0.01$, $\gamma_I = 0.05$, and $\gamma_O = 0.27$. From Fig. 1 we found that the segmentation by the proposed method is smoother and closer to the ground-truth than by the method by Feghali, so as the estimated optical flow field.

Two error metrics are adopted to compare the segmentation results to the ground-truth: the mean absolute difference estimates the averaged disagreement between the object boundaries. Another measure is the Jaccard similarity coefficient (Intersection/Union), which compares the areas of the segmented objects.

Fig. 1. Comparison results on synthetic image. (a) Original intensity image, (b) segmentation on noisy image by the method by Feghali, (c) segmentation on noisy image by the proposed method, (d) magnitude of motion field estimated from original intensity image, (e) magnitude of motion field estimated from noisy image by the method by Feghali, (f) magnitude of motion field estimated from noisy image by the proposed method.

The value ranges from 0 (no overlap) to 1 (total overlap), thus the higher value the better correlation. The measurement results against different level of noise are plotted in Fig. 2 and 3.

3.2 Neuronal Data

The cells in our neuronal images are ND7 cells, hybrid of mouse neuroblastoma and rat dorsal root ganglia. The purpose of the experiment is to characterize the dynamic properties of the filopodia. These highly mobile projections are continuously extended and retracted from the cell body. Cells were seeded on plastic petridishes and transfected with an expression plasmid for cherry-actin. The next day, cells were harvested and embedded in a collagen gel matrix (0.4mg/mL collagen) in a glass-bottomed petridish of 35mm diameter. One mL of DMEM (without phenol red) supplemented with 5% fetal calf serum was added to the dish after the gel has solidified. The petridish of cells was incubated on the

Fig. 2. Mean absolute differences at different noise levels on synthetic images

Fig. 3. Jaccard similarity coefficients at different noise levels on synthetic images

microscope for 30 minutes to stabilize the temperature and CO₂ before images were captured.

The imaging was performed on an Olympus IX81 equipped with a 60 $\times$ 1.20 UPLANSAPO objective. The images were acquired by a Photometrics QuantEM:512SC and the MetaMorph Imaging Software from Molecular Devices. The stack size of our 3D neuronal images is 281 $\times$ 233 $\times$ 10 voxels and the spatial

Fig. 4. Comparison results on cellular image. (a) First image of the temporal sequence, (b) segmentation by the method by Feghali, (c) segmentation by the proposed method, (d) second image of the temporal sequence, (e) magnitude of motion field estimated by the method by Feghali, (f) magnitude of motion field estimated by the proposed method.

resolution is $0.275 \times 0.275 \times 0.5 \mu\text{m}/\text{pixel}$. The temporal sequence contains 31 stacks, taken at an interval of 10 seconds.

The position of the cell is fixed but the cell morphology changes due to the dendrite growth and movement. In this case, the estimated motion field indicates the movement of each voxel within the cell. The parameter values for experiments on neuronal image are: $\alpha = 0$, $\lambda_I = 1$, $\lambda_O = 1$, $\beta = 0.01$, $\gamma_I = 0.02$ and $\gamma_O = 0.025$.

Fig. 4(a) and Fig. 4(d) show the middle sections from two successive stacks. The segmentation by the method by Feghali misses the cell boundary sometimes and contains singularities inside the cell. In contrast, the proposed method is more robust to the noise and finds the cell boundary accurately in spite of the low contrast; meanwhile, an accurate motion field is obtained.

4 Conclusion

We proposed a new method of joint tracking of cell morphology and motion from 3D temporal cellular images. Optical flow and region-based active contours methods are combined via a united energy formulation and solved iteratively. Motion estimation and segmentation are coupled directly so that the temporal dimension is taken into account. In addition, we make use of regional information in additional energy terms. The advantage of such method is that the segmentation relies on the average intensity of different regions rather than the presence of strong edge around object boundary. This increases the robustness and accuracy of our method. Experimental results on synthetic and real images demonstrate that the proposed method is able to give smooth and precise segmentation on noisy images with blurred boundary.

Another advantage of our method is that it is highly automatic. No human interference is required during the process, except that some parameter adjustments may be needed for the processing of a new dataset. Our method enables the quantitative analysis of cell morphology and motion from a large amount of 3D temporal cellular images. The method can help the automatic detection and separation of dendrites from cell body. The lengths of dendrites, the number of branches, and other quantitative features can be measured further from the segmentation results. The robustness and the accuracy of the method are vital for biological studies that make inferences on cell morphology and growth.

In our method, we assume that the motion of voxels within the cell is always larger than the motion of background. This assumption is roughly true since the motion is large near the cell boundary where growth is likely to occur. However, there could be near zero-motion regions inside the cell and this will cause a hole in the segmentation. In future studies, we will incorporate adaptive hole-filling algorithm in our method.

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