A VARIATIONAL MODEL FOR LEVEL-SET BASED CELL TRACKING
IN TIME-LAPSE FLUORESCENCE MICROSCOPY IMAGES

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ABSTRACT
Quantifying the motion and deformation of large numbers of cells through image sequences obtained with fluorescence microscopy is a recurrent task in many biological studies. Automated segmentation and tracking methods are increasingly needed to be able to analyze the large amounts of image data acquired for such studies. In addition, automated techniques have the possibility to improve sensitivity, objectivity, and reproducibility compared to human observers. Recent efforts in this area have revealed the potential of model evolution methods, notably active contours and level sets, for this purpose. One of the disadvantages of such methods is their sensitivity to parameter settings. In this paper we propose a variational model for level-set based cell tracking which involves less parameters with more intuitive meaning compared to previous approaches. The improved performance is demonstrated with experimental results on real time-lapse fluorescence microscopy image data.

Index Terms— Fluorescence microscopy, cell tracking, level sets, variational model.

1. INTRODUCTION

Cell motility and migration play a key role in many biological processes, such as fertilization, embryonic development, wound healing, immune response, and cancer metastasis. Understanding these complex processes is of major importance in developing procedures to combat diseases and to improve human health care [1]. In studying these processes, biologists increasingly rely on computerized methods for cell tracking, not only to cope with the sheer quantity of data to be analyzed, but also to improve sensitivity, objectivity, and reproducibility [2]. Existing cell tracking methods can roughly be divided into two main classes [3, 4]. The first class performs frame-by-frame cell detection followed by linking based on minimizing the distances between detected cells according to predefined criteria. The second class of approaches is based on mathematical shape models, which are first fit to the data, and then evolved in time to keep track of cell movements and deformations.

Model evolution approaches, based on active contours or level sets, are currently being explored by several groups and there is now a growing consensus that they are well suited to cell tracking. However, they also suffer from a number of shortcomings, in particular their sensitivity to parameter settings [2]. In this paper we propose an improved level-set based model evolution method for tracking cell movement and deformation in time-lapse fluorescence microscopy images. Specifically, we replace the classical model by Chan and Vese (CV), which has been used in recent approaches to cell tracking [5], by a variational model. Experiments on real fluorescence microscopy image data demonstrate that the latter model, which involves less parameters, yields superior segmentation and tracking results, even in the case of significant differences in average intensities of cells.

2. MATHEMATICAL MODEL

A classical level-set model for image segmentation is the one proposed by Chan and Vese [6]. This model is easy to implement and has recently been adapted to cell tracking [5] with promising initial results. However, its performance depends heavily on a number of parameters, some of which have no clear physical meaning, making it difficult to estimate in advance suitable values or even feasible limits for them. In practice these parameters are obtained experimentally. Another drawback of the model is that it fails in the case of multiple objects with large differences in average intensity, which occurs frequently in fluorescence microscopy. The multiphase level-set framework [7] can be used as a remedy in such cases, but is usually unfavorable due to the large increase in computational complexity compared to the single phase model. Here we propose to use a variational framework.

2.1. Variational Framework

In the variational framework of Rousson and Deriche [8], image segmentation is performed by minimizing an energy functional $E$, given by

$$E(\Omega) = \sum_{i=1}^{N} \int_{\Omega_i} -\log p(I(x)|\Omega_i)dx$$
$$+ \alpha \cdot \text{Length}(\partial \Omega) + \nu \cdot \text{Area}(\Omega), \quad (1)$$
where $\Omega_i$, $i = 1, \ldots, N$, are the regions (objects and background), $\Omega = \bigcup_{i=1}^N \Omega_i$ is the image domain, $\partial \Omega$ is the boundary between regions, $I(x)$ is the image intensity at position $x$, $\alpha$ and $\nu$ are positive parameters, and $p(I(x)|\Omega_i)$ is the a posteriori probability that pixel $x$ with intensity $I(x)$ belongs to the region $\Omega_i$. This distribution is also often called the feature distribution, which we assume here to be Gaussian, with mean $\mu_i$ and variance $\sigma^2_i$.

Representing the region boundary $\partial \Omega$ as the zero-level of the function $\phi(x)$, and considering the case when it is only necessary to separate foreground from background ($N = 2$), we can rewrite the energy functional (1) as

$$E(\phi, \Omega) = \int_{\Omega} [e_1(x)H_\epsilon(\phi) + e_2(x)(1 - H_\epsilon(\phi))] \, dx + \alpha \cdot \text{Length}(\partial \Omega) + \nu \cdot \text{Area}(\Omega),$$

where

$$e_i(x) = \log \left( \frac{\sigma_i^2}{\sigma^2} \right) + \frac{(I(x) - \mu_i)^2}{\sigma_i^2},$$

and

$$H_\epsilon(z) = \frac{1}{2} \left( 1 + \frac{2}{\pi} \arctan \left( \frac{z}{\epsilon} \right) \right)$$

is the regularized Heaviside function. Applying to (2) the Euler-Lagrange equation, we find the evolution equation

$$\phi_t(x) = \delta_\epsilon(\phi) \left[ \alpha \partial_x \left( \frac{\nabla \phi}{\sqrt{\nabla \phi}} \right) + e_2(x) - e_1(x) - \nu \right],$$

where $\delta_\epsilon(z) = H'_\epsilon(z)$ is the regularized Dirac function. While evolving the level-set function $\phi_t(x)$ from its initial position $\phi_0(x)$ according to (3), the Gaussian parameters $\mu_i$ and $\sigma_i$, $i = 1, 2$, are updated after every iteration according to

$$\mu_1 = \frac{\int_{\Omega} I(x)H_\epsilon(\phi(x)) \, dx}{\int_{\Omega} H_\epsilon(\phi(x)) \, dx},$$

$$\mu_2 = \frac{\int_{\Omega} I(x)(1 - H_\epsilon(\phi(x))) \, dx}{\int_{\Omega} (1 - H_\epsilon(\phi(x))) \, dx},$$

$$\sigma^2_1 = \frac{\int_{\Omega} (I(x) - \mu_1)^2 H_\epsilon(\phi(x)) \, dx}{\int_{\Omega} H_\epsilon(\phi(x)) \, dx},$$

$$\sigma^2_2 = \frac{\int_{\Omega} (I(x) - \mu_2)^2 (1 - H_\epsilon(\phi(x))) \, dx}{\int_{\Omega} (1 - H_\epsilon(\phi(x))) \, dx}.$$ 

The latter expressions are the solutions of the corresponding Euler-Lagrange equations for the unknown parameters $\mu_i$ and $\sigma_i$, $i = 1, 2$.

Since this variational model uses not only the intensity averages of the foreground and the background regions (as in the CV model), but also the intensity variances, it is able to properly segment cells even in the case of strong difference between their average intensities. Another advantage is that this model contains only two adjustable parameters (instead of four as in the CV model): $\alpha$, the weight of the length term in (2) corresponding to the curvature term in (3), and $\nu$, the weight of the area term.

2.2. Non-PDE Based Initialization

The final segmentation is obtained as the result of minimizing the energy functional (2) according to (3). The initial position $\phi_0(x)$ of the curve has a crucial influence on the number of iterations needed to reach the final result. The closer the cell is to the true object boundaries, the faster the optimal solution is found. As pointed out in [9], a non-PDE based technique is able to produce a segmentation which is sufficiently close to the local minimum. The idea behind this method is that starting from an arbitrary initial segmentation we consider each pixel separately and check what happens with the energy if we move this pixel from one class to another. If the energy decreases, then the pixel is moved to the new class, otherwise no changes occur. It turns out that, in the case when only the image-dependent energy is used, such a simple scheme converges really fast, typically within a few iterations.

In [5] it was suggested to use the non-PDE based segmentation in the following fashion. First, the algorithm is applied with image-dependent energy only (a few iterations). Next, the algorithm is applied with both smoothness- and image-dependent energies (also a few iterations only). Here we use a slightly modified approach. First, we check how the image-dependent energy of every pixel changes if we move it from one class to another. Next, after all pixels are distributed between classes according to the calculated energy difference, we do the same for the smoothness-based energy (within the same iteration). Using this approach, the method usually converges within a few iterations. The other difference compared to the original model [9] is the fact that we use a different energy function. The expressions for the energy differences and their derivation are given in the Appendix.

3. TRACKING ALGORITHM

For tracking cells in time-lapse fluorescence microscopy image sequences we use the method from [5] but with the new image-based energy. The main steps of the scheme are:

1. Segmentation of the first image of the stack using the variational model (Section 2.1). The initial position of the level-set function is obtained from non-PDE based segmentation (Section 2.2).
2. Extract each cell from the segmented initial image and initialize its corresponding level-set function \( \phi_{i,0}(x) \) to the signed distance function of the cell’s boundary.

3. At every time step, minimize the total energy corresponding to all \( N \) level-set functions (here \( N \) is equal to the number of detected cells). In our model the total energy is given by the functional

\[
E(\phi_1, \ldots, \phi_n) = \int_{\Omega} \sum_{i=1}^{N} \left[ \alpha \delta(\phi_i) |\nabla \phi_i| \right. \\
+ H_\varepsilon(\phi_i) e_{1,i}(x) + \frac{1}{N} \prod_{j=1}^{N} (1 - H_\varepsilon(\phi_j)) e_2(x) \\
+ \gamma \sum_{i<j} H_\varepsilon(\phi_i) H_\varepsilon(\phi_j) \right] dx,
\]

where \( e_{1,i}(x) \) is the “internal” energy of \( \phi_i \).

The evolution equation for each of the level set functions is obtained from (4) using the Euler-Lagrange equation. Here we use the narrow-band technique in order to speed-up the calculations, and we reinitialize all level-set functions to the signed distance function after a certain number of iterations. For the reinitialization we have chosen the fast subvoxel reinitialization algorithm suggested in [10] for the reason that it can be combined naturally with the narrow-band technique. The segmentation result for each image in the sequence is used as initialization for the next image.

4. EXPERIMENTAL RESULTS

In order to compare the performance of the CV and the variational model we applied both models on different sets of real fluorescent microscopy image sequences. We observed that the variational model was able to segment and track cells correctly in all cases, while the CV model failed to segment cells with strongly varying intensities. We note that in fluorescence microscopy image data such intensity variations (in both space and time) often occur due to photobleaching. A representative example of segmentation using both models is shown in Figure 1. It is clear that for the same values of weights for the smoothness- and area-terms, the CV model produces incorrect results, while the results obtained with the variational model are correct. For this particular example it was possible to improve the segmentation for the CV model by using a much higher value for the parameter \( \mu \). In practice, however, such high values for the curvature-term weight are undesirable since they may lead to numerical instabilities. In the case of more complicated images we found that it was impossible to choose a combination of weights for the CV model that would produce a correct segmentation. By contrast, the variational model yielded correct results in those images. A sample result of tracking an image sequence is shown in Figure 2. As can be seen, the model is able to handle cell division correctly, although it encounters difficulties if the distance between positions of a cell in two consecutive frames exceeds the cell’s diameter.

5. DISCUSSION

In this paper we have argued and demonstrated the advantages of using the variational model [8] for level-set based tracking in time-lapse fluorescence microscopy image data.
This model is a generalization of the classical CV model and it produces correct segmentations even in cases where the CV model fails (for example for images with considerable differences in average cell intensities). An additional advantage of the variational model in comparison with the CV model is that it has less parameters to be tuned, which makes the final result less sensitive to different combinations of parameter values. Using the new image-based energy term, we reformulated the tracking scheme from [5] and tested it on real fluorescence microscopy image data. In order to obtain a fast segmentation of the first image of the sequence we made use of the non-PDE based technique [9], which we also reformulated to incorporate the new image-based energy. While we have shown only 2D image sequences in this paper for illustration, the proposed method can be straightforwardly extended to 3D image sequences.

6. REFERENCES


APPENDIX

Let us assume that our image consists of two classes, $A$ and $B$, with $m$ and $n$ the number of pixels in these classes, respectively. From (2) we calculate the image-based energy term for this case as

$$E_{im} = m \log (\sigma_A^2) + \frac{1}{\sigma_A^2} \sum_{i=1, x_i \in A}^m (I(x_i) - \mu_A)^2$$

$$+ n \log (\sigma_B^2) + \frac{1}{\sigma_B^2} \sum_{i=1, x_i \in B}^n (I(x_i) - \mu_B)^2,$$

with

$$\mu_A = \frac{1}{m} \sum_{i=1, x_i \in A}^m I(x_i), \quad \mu_B = \frac{1}{n} \sum_{i=1, x_i \in B}^n I(x_i),$$

$$\sigma_A^2 = \frac{1}{m} \sum_{i=1, x_i \in A}^m (I(x_i) - \mu_A)^2,$$

$$\sigma_B^2 = \frac{1}{n} \sum_{i=1, x_i \in B}^n (I(x_i) - \mu_B)^2.$$

Assume now that pixel $x_0$ with intensity $I_0$ is moved from class $A$ to class $B$. The difference between the new image-based energy and the previous one is

$$\Delta E_{12} = n \log \left( \frac{\Sigma_B^2}{\Sigma_B^2} \right) + m \log \left( \frac{\Sigma_A^2}{\Sigma_A^2} \right) + \log \left( \frac{\Sigma_B^2}{\Sigma_A^2} \right),$$

where

$$\Sigma_A^2 = \frac{m}{m - 1} \left[ \sigma_A^2 - \frac{(I_0 - \mu_A)^2}{m - 1} \right]$$

and

$$\Sigma_B^2 = \frac{n}{n + 1} \left[ \sigma_B^2 + \frac{(I_0 - \mu_B)^2}{n + 1} \right]$$

are the new variances. In case when the pixel moves from class $B$ to class $A$, the energy difference is

$$\Delta E_{21} = n \log \left( \frac{\Sigma_B^2}{\Sigma_A^2} \right) + m \log \left( \frac{\Sigma_A^2}{\Sigma_B^2} \right) - \log \left( \frac{\Sigma_B^2}{\Sigma_A^2} \right),$$

and the new variances are

$$\Sigma_A^2 = \frac{m}{m + 1} \left[ \sigma_A^2 + \frac{(I_0 - \mu_A)^2}{m + 1} \right],$$

$$\Sigma_B^2 = \frac{n}{n - 1} \left[ \sigma_B^2 - \frac{(I_0 - \mu_B)^2}{n - 1} \right].$$