Cell Segmentation Using Level Set Methods with a New Variance Term

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Abstract. We present a new method for segmentation of phase-contrast microscopic images of cells. The algorithm is based on the variational formulation of the level set method, i.e. minimizing of a functional, which describes the level set function. The functional is minimized by a gradient flow described by an evolutionary partial differential equation. The most significant new ideas are initialization using thresholding and the introduction of a new term based on local variance that speeds up convergence and achieves more accurate results. The proposed algorithm is applied on real data and compared with another algorithm. Our method yields an average gain in accuracy of 2 %.

Keywords: Segmentation · Level set method · Active contours · Phase contrast microscopy

1 Introduction

Live cell imaging captures crucial information of many biological processes with direct implication for human health [1]. The analysis of our microscopic images is used for the development of body implants, see [2]. The human body is very sensitive to foreign materials, unsuitable implants may cause immune reactions. Therefore, the biocompatibility or biotoxicity of various materials are studied.

The images we process come from experiments in vitro using cancer cells due to their resistance and easy laboratory preservation. The cells are scanned with a phase-contrast microscope at regular time intervals, in our case every 2 min. The images are analyzed to determine the rate of cell growth, which leads to the problem of segmentation of cells from the background.

The main goal is to find an efficient algorithm since the segmentation is usually performed manually, which is a tedious and time consuming work. Automatic segmentation is a complicated problem in itself and in our case there are several factors that make our task even more difficult. The microscopic images contain artifacts like halos, bright areas around the cell borders. The colour of the background is inconveniently similar to the colour of the cell interiors. The microscopic images also suffer from poor focus and impurities.

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There are various segmentation approaches in the literature, e.g. machine learning [3] or motion detection [2]. In this paper, we focus on the method of active contours, first introduced in [4], and its level set formulation proposed in [5]. We use the variational formulation by Li *et al.* [6] which does not need re-initialization. We introduce a new term into the formulation to improve the method for microscopic images and we propose an efficient initialization.

The remainder of this paper is organized as follows: the first part of Sect. 2 describes the variational formulation proposed by Li, in the second part we introduce our new term and propose a new initialization. In Sect. 3 we describe implementation. We validate our approach in Sect. 4 and conclude in Sect. 5.

2 Level Set Method

The level set method is a simple and versatile method for numerical analysis of the motion of a finite set of closed curves that divides the plane into exterior and interior regions, i.e. the background and the foreground.

A zero level set of a function ϕ is a set where the values of the function are equal to zero: $L_\phi(t) = \{(x, y)|\phi(t, x, y) = 0\}$. The main idea behind the level set method is that a curve can be seen as the implicitly given zero level set of a function in higher dimension. The goal is to capture and analyze the motion of the curve in time. Instead of moving the curve itself, we will be evolving the level set function.

2.1 Variational Formulation of the Level Set Method

In this section we describe the functional proposed by Li *et al.* [6]. An explicit energy functional $E(\phi)$ will be defined, so that the zero level curve of the minimizer ϕ captures the desired features in an image, in our case the cell edges.

The energy functional has two parts:

$$E(\phi) = E_m(\phi) + \mu P(\phi),$$

where $\mu > 0$ is a weighting parameter. The energy $E_m(\phi)$ depends on the image data, therefore it is called the external energy. The energy $P(\phi)$ is a function of ϕ only and is called the internal energy.

Internal Energy. In implementations of traditional level set methods, it is numerically important to maintain the level set function close to a signed distance function [7]. By definition, a signed distance function f must satisfy

$$|\nabla f(\mathbf{x}, t)| = 1 \quad \text{a.e. } \mathbf{x} \in \Omega,\ \forall t, \tag{1}$$

where $\Omega \subset \mathbb{R}^2$ is the image support.

One approach is a periodic re-initialization of the level set function. However, this technique has undesirable side-effects and it still remains a serious question when to apply the re-initialization process. Li *et al.* [6] introduced a new internal energy term that penalizes the deviation of the level set function from the signed distance function property (1):

$$P(\phi) = \int_{\Omega} \frac{1}{2} \left(|\nabla \phi| - 1 \right)^2 dxdy. \tag{2}$$

Therefore, re-initialization is no longer necessary.

External Energy. Let I be an image. The edge indicator function is defined as $g = \frac{1}{1+|\nabla(G_\sigma * I)|^2}$, where G_σ is the Gaussian kernel with standard deviation σ and $*$ denotes convolution.

The external energy is defined as

$$E_m(\phi) = \lambda L(\phi) + \alpha A(\phi), \tag{3}$$

where $\lambda > 0$, α are constants and the terms $L(\phi)$ and $A(\phi)$ are

$$L(\phi) = \int_{\Omega} g\delta(\phi)|\nabla\phi| dxdy, \quad A(\phi) = \int_{\Omega} gH(-\phi)dxdy, \tag{4}$$

where δ is the delta distribution and H is the Heaviside function:

$$\int_{-\infty}^{\infty} \delta(x)dx = 1, \quad H(x) = \begin{cases} 1, \, x \geq 0, \\ 0, \, x < 0. \end{cases} \tag{5}$$

It is well known, [8], that the geometrical meaning of the energy $L(\phi)$ is the length of the zero level curve of the level set function ϕ and therefore adding it has a smoothing effect on the zero level curve. The energy functional $A(\phi)$ minimizes the area of the region inside the zero level curve and is proposed to speed up the curve evolution. In our case the initial contour will be placed outside the cells, therefore the coefficient α in (3) should be positive.

The total energy functional is defined by

$$\begin{aligned} E(\phi) &= \mu P(\phi) + E_m(\phi) \qquad (6) \\ &= \mu \int_{\Omega} \frac{1}{2} \left(|\nabla\phi| - 1 \right)^2 dxdy + \lambda \int_{\Omega} g\delta(\phi)|\nabla\phi| dxdy + \alpha \int_{\Omega} gH(-\phi)dxdy. \end{aligned}$$

Gradient Flow Formulation. We seek the stationary solution of the energy functional E given by (6), which is computed using its Euler-Lagrange equation. The gradient flow that minimizes E is the following evolutionary equation:

$$\frac{\partial \phi}{\partial t} = -\frac{\partial E}{\partial \phi} = -E',$$

which is equivalent to solving the variational problem with the steepest-descent method.

To explicitly express the gradient flow we apply the Euler-Lagrange equation to the functional (6). The gradient flow is then defined by

$$\frac{\partial \phi}{\partial t} = \mu \left[\Delta\phi - \operatorname{div}\left(\frac{\nabla\phi}{|\nabla\phi|} \right) \right] + \lambda\delta(\phi)\operatorname{div}\left(g\frac{\nabla\phi}{|\nabla\phi|} \right) + \alpha g\delta(\phi). \tag{7}$$

2.2 New Variational Term in the Gradient Flow

To improve the accuracy and speed up the evolution we have incorporated a new term into the gradient flow (7).

Since our application is the segmentation of cell images, we can assume the images will have similar features. An image may contain undilated cells. A typical undilated cell is shown on the left of Fig. 1. The cell center is of a similar colour as the background and forms a visual edge inside the cell. As a consequence, a contour may form inside the cell, which is shown in the right image of Fig. 1.

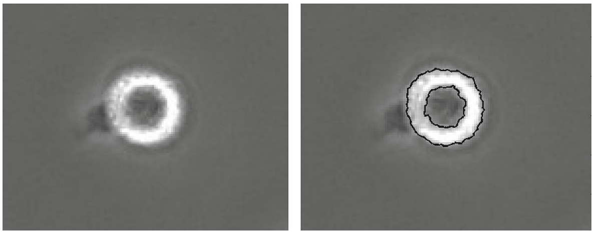

Fig. 1. Left image: image of an undilated cell. Right image: an undesired contour inside a cell that may form using Li's method.

To avoid the undesired interior contour, we introduce a new term to the gradient flow (7), which we call the variance term V. It is based on the assumption that the local variance of the background is significantly smaller than variance of the cells. The variance term uses the mean values of variance of areas currently assigned as background, denoted as v_1, and objects, denoted as v_2. Consequently, it drives the motion of the level set curve of ϕ towards one of them, depending on whether the variance of a point is more similar to either v_1 or v_2. The variance term is defined as

$$V(\phi) = \rho\delta(\phi)\left(-\left(Var - v_1\right)^2 + \left(Var - v_2\right)^2\right), \tag{8}$$

where $\rho > 0$ is a weighting parameter, Var is the variance of the image on a neighbourhood of a given point, v_1 is the mean exterior variance and v_2 is the mean interior variance. The delta distribution selects only the zero level curve from the domain of ϕ.

In contrast to Li's method, we do not use the edge indication function based on experimental results on our data.

The new gradient flow is then defined by

$$\begin{aligned}\frac{\delta\phi}{\delta t} = \mu\left[\triangle\phi - \operatorname{div}\left(\frac{\nabla\phi}{|\nabla\phi|}\right)\right] + \lambda\delta(\phi)\operatorname{div}\left(\frac{\nabla\phi}{|\nabla\phi|}\right) + \\ + \alpha\delta(\phi) + \rho\delta(\phi)\left(-\left(Var - v_1\right)^2 + \left(Var - v_2\right)^2\right).\end{aligned} \tag{9}$$

2.3 New Initialization of the Level Set Function by Variance

In the traditional level set formulation it is important to initialize the level set function ϕ as a signed distance function, [7]. Due to the new penalizing term (2) we can use more general functions in our formulation. The region based initialization proposed by Li *et al.* [6] is flexible for various applications.

As we process a sequence of images, a natural candidate for the initialization is the resulting level set function from the previous image. However, this method fails because the initial curve can partially lie inside a cell, which violates the assumption for the parameter of the area term (4).

Therefore we suggest a different approach. Our method utilizes the same property as when we introduced the variance term (8), i.e. the assumption that the background has significantly smaller variance than the cells. For each pixel we compute the variance of its neighborhood and then we use thresholding on the image of variance. This initialization provides fast convergence after a relatively small number of iterations (150 iterations for our images, cf. Sect. 4).

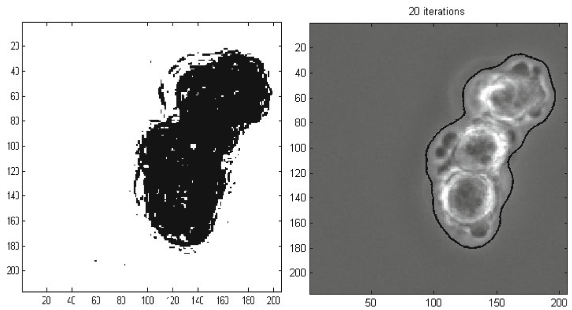

Fig. 2. Left image: initialization by thresholding the image of variance. Right image: a smooth curve after 20 iterations of our algorithm using the initialization by variance.

As an example, we use an image of a cell cluster. The first image in Fig. 2 shows the binary image obtained by thresholding. A method for optimizing the value of the threshold is described in Sect. 3.2. Although the initial level set function ϕ_0 has many zero level curves for only one cell cluster, due to the formulation of the gradient flow the small curves disappear after less than 20 iterations, see the second image in Fig. 2.

3 Implementation

3.1 Numerical Scheme

In practice, the delta distribution and the Heaviside function (5), $\delta(x) = H'(x)$, are numerically regularized [6]:

$$\delta_\epsilon(x) = \begin{cases} 0, & |x| > \epsilon, \\ \frac{1}{2\epsilon}\left[1 + \cos\left(\frac{\pi x}{\epsilon}\right)\right], & |x| \le \epsilon. \end{cases}$$

For all our experiments we set the parameter ϵ to 1, which is the pixel size.

The spatial derivatives are approximated by central differences. The time derivative is approximated by the forward difference with time step τ.

The level set function ϕ is discretized as $\phi^k_{i,j}$, where (i, j) is are space indexes and k is a time index. Then the level set evolution using the iterative gradient flow (9) can be written as the process:

$$\phi^{k+1}_{i,j} = \phi^k_{i,j} + \tau F(\phi^k_{i,j}),$$

where $F(\phi^k_{i,j})$ is the approximation of the right hand side of (9).

3.2 Automatic Optimization of the Weighting Parameters

We used manual optimization of the parameters in our initial experiments. However, this method of optimizing can be tedious. Therefore, we tried several automatic methods of optimization in MATLAB to see whether the automatic methods are applicable to our problem.

The most accurate results were obtained by the trust-region-reflective method, see [9]. The method minimizes the difference between the results of the segmentation and the ground truth, i.e. images segmented manually by specialists.

We applied the automatic optimization only to the first image of the sequence and then used the resulting parameters for all of the remaining images. This process simulates the supposed use on real data, where only the ground truth of the first image is needed. The parameter ρ for our new variance term (8) was optimized separately.

The values of the parameters we used in our experiments are

$$\mu = 0.2,\ \lambda = 6.9913,\ \alpha = 0.33,\ \rho = 0.008,\ p = 2.0439, \tag{10}$$

where the first four parameters are the weighting parameters in the gradient flow (9) and the last parameter is the threshold used in initialization by variance.

4 Results

Our images come from the Laboratory of Tissue Culture, University of South Bohemia in České Budějovice, Czech Republic.

The sequence we process has 2161 images. Images are taken in 2 min interval. Since we need manually segmented images from specialists as the ground truth and since it is time consuming to produce them, we chose 49 equidistantly distributed images from this sequence to analyze. The images were segmented by our algorithm and compared with results arising from the algorithm by Li *et al.*, described in Sect. 2. We used our initialization by variance for both methods, which ensures convergence after 150 iterations for both algorithms. For Li's algorithm we used the same parameters specified in (10). We use the F_1 score for evaluation (the highest value being 1).

Table 1. Comparison of the mean values of F_1 scores of 49 images of our method, Li's method and our initialization – thresholding of the variance image.

F_1 score	Our algorithm	Li's algorithm	Our initialization
Original images	0.8901	0.8679	0.8415
Cropped images	0.8955	0.8720	0.8455

We computed the mean value of the F_1 scores of the 49 images for each algorithm and for the initialization by variance which is shown in Table 1. The threshold in the initialization by variance was chosen as the result of the automatic optimization (10). One problem of our images is embedded text at the corners of the images. We therefore evaluate the F_1 score also on cropped images without the text at the corners. Table 1 shows that our initialization by variance is already very efficient. The mean value of the F_1 score of our method is more than 2 % higher than Li's which is a considerable improvement taking into account the current high accuracy level and the inaccuracy of the manual segmentation.

Figure 3 demonstrates the robustness of our algorithm on one of the first images and on the last image of the sequence. There are isolated cell clusters on a background in the first image while the majority of the last image is covered with cells. The black curves represent the zero level set and separates the image into cells and background.

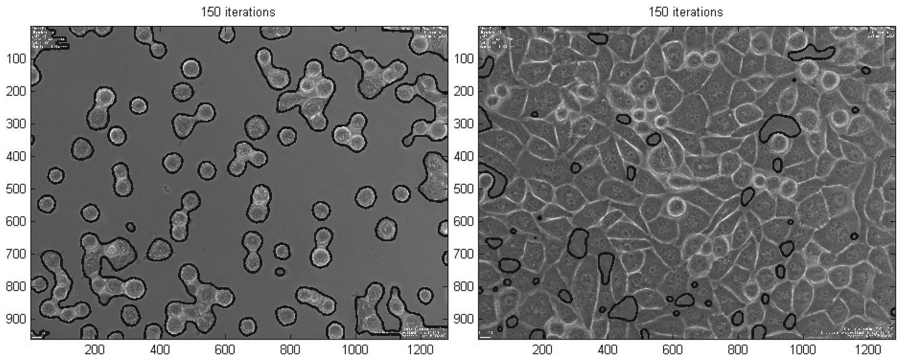

Fig. 3. Resulting segmentation of one of the first images of the sequence (left) and of the last image of the sequence (right).

5 Conclusion

In this paper we propose an improved variational level set method for segmentation of microscopic images of cells. A new term based on variance was incorporated into the functional and using the edge indicator function was omitted for our images. We also introduce an efficient region-based initialization. The results of the proposed algorithm are compared with the original method. We suppose that the method can be used generally for segmenting textures, e.g. grass from buildings or the sky.

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