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VARIATIONAL LEVEL-SET WITH GAUSSIAN SHAPE MODEL FOR CELL SEGMENTATION

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Abstract

In analysis of microscopy based images, a major challenge lies in splitting apart cells that appear to overlap because they are too densely packed. This task is complicated by the physics of the image acquisition that causes large variations in pixel intensities. Each image typically contains thousands of cells with each cell having a different orientation, size and intensity histogram. In this paper, a spatial intensity model of a nucleus is incorporated into [1] to aid cell segmentation from microscopy datasets. An energy functional is defined and with it the spatial intensity distribution of a nuclei is modeled as a Gaussian distribution with constant intensity background. Experimental results on a variety of microscopic data validate its effectiveness.

Index Terms

shape model; level-sets; intensity distribution

1. INTRODUCTION

In microscopy image analysis, segmentation and tracking of cells/nuclei form the preliminary as well as the most significant steps in any analysis protocol. Normally, cells appear tightly packed and have deformable membranes making the task of splitting them quite complicated. Each cell usually has a different orientation, shape and size. The process of acquisition involves injecting a tissue with a fluorescent marker and exciting it with radiation and observing their emission under a microscope. Each nucleus absorbs a different amount of the stain and hence are characterized by a non-constant intensity profile. Please refer to Fig. 1. Therefore, simple segmentation strategies based on thresholding, morphological operators and edge-based techniques are rendered useless.

We present the problem of modeling a nucleus intensity by a distribution. Consider the ideal situation where the fluorescent marker would be uniformly distributed inside the 3D volume of individual nucleus. Suppose that the imaging process did not induce any noise. That would result in a constant intensity profile. However, the process of imaging is equivalent to a convolution operation performed on the underlying piecewise-constant intensity field using the point-spread-function of the microscope. Assuming a parametric shape model of a nucleus and applying a simple point-spread function (PSF) model leads to a very complicated model containing high order terms. Furthermore, the PSF of a microscope varies depending on the objective used, the setting for the size of the confocal aperture, and can be distorted by inhomogeneities in the specimen itself. Hence, a rigorous model of the nucleus is quite difficult to develop.

The segmentation of overlapping nuclei in a biologically consistent manner is an active topic of research. Tscherepanow *et al.* [2] presented an active contour approach with a *snakes*

formulation wherein overlapping cells are segmented as dictated by a 2D elliptical model. Jones *et al.* [3] presented a novel method that first defines a metric in the image plane for calculating distances from seed regions. Pixels are then assigned to cells according to their distance from the corresponding nucleus under that metric. Mosaliganti *et al.* [4] used geodesic active contours with shape priors for nuclei segmentation. They however failed to model the shape as a consequence of intensity distribution. In a more recent and relevant approach, Khairy *et al.* [5] formulate the image model as stochastically generated based on biological priors and physics of image formation. Since cell nuclei are membrane-bound their shapes are subject to membrane lipid bilayer bending energy, which are used to constrain individual contours. However, they still neglect the spatial distributions.

In our approach, we define an energy functional that describes the image as a constant intensity background and a nucleus that has a spatial intensity distribution that fits Gaussian distribution. An ideal segmentation is obtained by a contour that separates these two intensity distributions. By representing the contour as a level-set function, a variational level-set is derived to minimize the energy functional. Our level-set formulation can be considered as an extension of the Chan and Vese method [1] to segment images with piecewise constant image regions. Using our approach, an effective segmentation of nuclei may be obtained that takes into account their intensity profiles and can be used in conjunction with other constraints on volume, shape and smoothness to obtain tangible segmentations.

The rest of the paper is organized as follows. Section 2 provides some background information on the level-set methodology applying the Chan and Vese method [1]. This is essential to place our method into perspective. In Section 3, we formulate the energy functional for our images in the context of active contour models and derive the update equation. We describe extensions to the case where multiple cells/nuclei are present in the images. Section 4 showcases our results on zebrafish cell images obtained from a confocal microscope and reports on validation efforts. Finally, in Section 5, we provide a summary and describe our plans for the future.

2. BACKGROUND

2.1. Level-set formalism

Consider a finite domain $\Omega \supseteq \mathbb{R}^d$. In the level-set formalism, one interface Γ is represented as the zero level-set of a Lipschitz continuous function $\varphi: \Omega \rightarrow \mathbb{R}$ satisfying:

$$\text{if } \begin{cases} \varphi(\mathbf{p}) > 0, & \mathbf{p} \text{ belongs to the inner part } \Omega_{in}, \\ \varphi(\mathbf{p}) < 0, & \mathbf{p} \text{ belongs to the outer part } \Omega_{out}, \\ \varphi(\mathbf{p}) = 0, & \mathbf{p} \text{ is on the interface } \Gamma. \end{cases}$$

where Ω_{in} is a region in Ω bounded by Γ , and Ω_{out} is defined as the complement of Ω_{in} , *i.e.* $\Omega_{out} = \Omega - \Omega_{in}$.

2.2. Variational region-based formulation

The principle of variational level-set methods is to minimize a given functional which aims to extract one, or several, element(s) of interest from the background. A general expression of such a functional can be formulated [6] as the sum of an interface energy criterion (often referred as *contour-term*) and internal and external energy criteria (often referred as *region-term*). One very popular example of such a formulation has been introduced by Chan and Vese [1] and aims to partition an image into regions with constant piecewise constant intensity:

$$J[\varphi] = \lambda_{in} \int_{\Omega} (I(\mathbf{p}) - c_{in})^2 H_{\varepsilon}(\varphi(\mathbf{p})) d\mathbf{p} \\ + \lambda_{out} \int_{\Omega} (I(\mathbf{p}) - c_{out})^2 (1 - H_{\varepsilon}(\varphi(\mathbf{p}))) d\mathbf{p} \\ + \mu \int_{\Omega} \delta_{\varepsilon}(\varphi(\mathbf{p})) \cdot \|\nabla \varphi(\mathbf{p})\| d\mathbf{p} + \nu \int_{\Omega} H_{\varepsilon}(\varphi(\mathbf{p})) d\mathbf{p}$$

where I is the image to be segmented, φ is the implicit function, $\delta_{\varepsilon}(\cdot)$ and $H_{\varepsilon}(\cdot)$ are respectively some regularized versions of the Dirac and Heaviside functions (see Eq. 1), c_{in} and c_{out} are constants calculated below and λ_{in} , λ_{out} , μ , ν are some given hyper-parameters. Note that the first term corresponds to an internal region criterion energy, the second one to an external region criterion energy, and the two last ones refer respectively to the hyper-area of the interface and the hyper-volume of the inner part.

$$H_{\varepsilon}(x) = \frac{1}{2} + \frac{1}{\pi} \arctan\left(\frac{x}{\varepsilon}\right); \delta_{\varepsilon}(x) = \frac{\varepsilon}{\pi(\varepsilon^2 + x^2)} \quad (1)$$

c_{in} and c_{out} are iteratively computed as follows:

$$c_{in} = \frac{\int_{\Omega} I(\mathbf{p}) H_{\varepsilon}(\varphi(\mathbf{p})) d\mathbf{p}}{\int_{\Omega} H_{\varepsilon}(\varphi(\mathbf{p})) d\mathbf{p}} \quad (2)$$

$$c_{out} = \frac{\int_{\Omega} I(\mathbf{p}) (1 - H_{\varepsilon}(\varphi(\mathbf{p}))) d\mathbf{p}}{\int_{\Omega} (1 - H_{\varepsilon}(\varphi(\mathbf{p}))) d\mathbf{p}} \quad (3)$$

3. OUR PROPOSAL

By analyzing the spatial intensity distribution of a single nucleus, we propose a model in 3D (see Section 3.1). This model is integrated into a new energy functional for the cell segmentation that is solved by using the level-set procedure (see Section 3.2). The minimization of this new functional is performed by iteratively updating an implicit function and calculating the intensity model parameters. To segment thousands of cells, we then use a multiphase extension similar to the one proposed by Vese and Chan [7] (see Section 3.3).

3.1. Intensity model

We first analyze images at two scales: the embryo scale and the cell scale.

When looking at the embryo scale, the image intensity distribution of the background can be approximated as a constant value with some additive Poisson noise. For the sake of simplicity, in this paper we will consider that the Poisson noise is negligible. The background can thus be approximated as a constant value.

When looking at the cell scale (see Fig. 1(a)), and more precisely at profiles of the image intensity of given cells (see Fig. 1(b)), in any direction the profile can be approximated as a gaussian kernel with different variance. The overall profile in 3D can be approximated by a centered gaussian kernel:

$$K(\mathbf{p}) = a \cdot \exp\left(-(\mathbf{p} - \mathbf{c})^t \cdot \mathbf{B} \cdot (\mathbf{p} - \mathbf{c})\right) \quad (4)$$

where $\mathbf{c}$ is the center of the cell, $\mathbf{B}$ is a 3×3 positive-definite matrix which defines the orientation and the size of the cell, and a is a positive constant.

3.2. Single cell segmentation

Inspired from the formulation of Chan and Vese [1] (see Section 2.1), we propose a new functional in which we incorporate our intensity model:

$$\begin{aligned} J[\varphi] = & \lambda_{out} \int_{\Omega} (I(\mathbf{p}) - c_{out})^2 (1 - H_{\varepsilon}(\varphi(\mathbf{p}))) d\mathbf{p} \\ & + \lambda_{in} \int_{\Omega} \left(\ln \left(\frac{I(\mathbf{p})}{K(\mathbf{p})} \right) \right)^2 H_{\varepsilon}(\varphi(\mathbf{p})) d\mathbf{p} \\ & + \mu \int_{\Omega} \delta_{\varepsilon}(\varphi(\mathbf{p})) \cdot \|\nabla \varphi(\mathbf{p})\| d\mathbf{p} + \nu \int_{\Omega} H_{\varepsilon}(\varphi(\mathbf{p})) d\mathbf{p} \end{aligned} \quad (5)$$

where $K(\mathbf{p})$ is a centered anisotropic gaussian kernel (see Eq. 4), λ_{in} and λ_{out} are given hyper-parameters. The third and fourth terms represent the length and area regularization terms weighted by the coefficients μ and ν .

Using Euler-Lagrange principle, it is easy to show that the minimization of the functional (Eq. 5) is given by the level-set evolution:

$$\begin{aligned} \frac{\partial \varphi}{\partial \tau} = & \delta_{\varepsilon}(\varphi) \left[\mu \cdot \operatorname{div} \left(\frac{\nabla \varphi}{\|\nabla \varphi\|} \right) - \nu - \lambda_{in} \cdot \left(\ln \left(\frac{I(\mathbf{p})}{K(\mathbf{p})} \right) \right)^2 + \lambda_{out} \cdot (I(\mathbf{p}) - c_{out})^2 \right] \\ \varphi(\mathbf{p}, 0) = & \varphi_0(\mathbf{p}) \end{aligned}$$

where c_{out} and the parameters of the centered anisotropic gaussian kernel ($a, \mathbf{c}, \mathbf{B}$) are computed at each iteration. φ_0 is the initial implicit function.

For the sake of simplicity, we redefine $\ln(K(\mathbf{p}))$ as follows:

$$\begin{aligned} \ln K(\mathbf{p}) = & m_0 + m_1 x + m_2 y + m_3 z + m_4 x^2 + m_5 xy + m_6 xz + m_7 y^2 + m_8 yz + m_9 z^2 \\ = & \mathbf{m} \cdot \mathbf{u}^t(\mathbf{p}) \end{aligned} \quad (6)$$

where $\mathbf{p} = (x, y, z)$, $\mathbf{m}$ is the coefficient vector, and $\mathbf{u}(\mathbf{p})$ is the trivariate monomial vector of order 2, i.e. $\mathbf{u}(\mathbf{p}) = \{x^i y^j z^k; i + j + k \leq 2\}$.

At each iteration with a given implicit function φ , we need to re-calculate $\mathbf{m}$ and c_{out} in order to minimize the functional in Eq. 5. The minimization of the first term in Eq. 5 leads to the analytical expression of c_{out} given in Eq. 3. It can easily be shown that the minimization of the second term in Eq. 5 leads to a discrete least-squares problem:

$$\begin{aligned} \mathbf{m}^* = & \arg \min_{\mathbf{m}} \int_{\Omega} \left(\ln \left(\frac{I(\mathbf{p})}{K(\mathbf{p})} \right) \right)^2 H_{\varepsilon}(\varphi(\mathbf{p})) d\mathbf{p} \\ = & \arg \min_{\mathbf{m}} \sum_{\mathbf{p}_k \in \Omega} (\ln(I(\mathbf{p}_k)) - \mathbf{m} \cdot \mathbf{u}^t(\mathbf{p}_k))^2 \end{aligned}$$

The solution is easily calculated via the normal equation:

$$\mathbf{m}^* = (\mathbf{M}^t \cdot \mathbf{M})^{-1} \cdot \mathbf{M}^t \cdot \mathbf{y} \quad (7)$$

where $\mathbf{M}$ is a matrix of size $10 \times n$ with $M_{ij} = \mathbf{u}_i(\mathbf{p}_j)$ (n is the number of pixels in the inner part); $\mathbf{y}$ is a vector of size n with $y_i = \ln(I(\mathbf{p}_i))$. In our implementation, at each iteration in the level-set evolution, we solve a linear system, via a Singular Value Decomposition, to compute the coefficients $\mathbf{m}^*$ (Eq. 7). Note that $\mathbf{m}$ is directly connected (Eq. 6) to the coefficients a , B and c of the anisotropic gaussian kernel (Eq. 4).

3.3. Multiple cells segmentation

In order to extract several cells in the same time, we provide a *multiphase* extension of the previous method (see section 3.2) in the spirit of [7].

Consider N level set functions $\{\varphi_1, \dots, \varphi_N\}$ and intensity parameters $\{\mathbf{m}_1, \dots, \mathbf{m}_N\}$ (see Eq. 4–6) respectively and c_{out} represents the background intensity; N parameters $\{\lambda_{1,in}, \dots, \lambda_{N,in}\}$ are scalar weights of the individual object intensity fitting terms and λ_{out} is the weight for the background intensity fitting term. We finally incorporate length and area regularization terms and an overlap penalty function term, weighted via the parameters μ , ν and γ respectively.

Using Euler-Lagrange principle, we get for the i -th level set function, the following evolution equation:

$$\frac{\partial \varphi_i}{\partial \tau} = \delta_\varepsilon(\varphi_i) \left[\mu \cdot \operatorname{div} \left(\frac{\nabla \varphi_i}{\|\nabla \varphi_i\|} \right) - \nu - \gamma \sum_{j \neq i} H_\varepsilon(\varphi_j) + \lambda_{out} \prod_{j \neq i} H_\varepsilon(\varphi_j) (I(\mathbf{p}) - c_{out})^2 - \lambda_{i,in} \left(\ln \left(\frac{I(\mathbf{p})}{K(\mathbf{p})} \right) \right)^2 \right]$$

4. RESULTS

Here, we present results of our method on three kinds of data: a synthetic nucleus image, a real nucleus image, and a real data set. Our data consist of 3D+t images of zebrafish development acquired from a confocal microscope. A typical time-point in the dataset is obtained every 2 minutes and has pixel dimensions of $1024 \times 1024 \times 80$. Each voxel has physical dimensions of $0.2 \times 0.2 \times 1 \mu\text{m}$. In this paper, we process a given time point at an early stage of the zebrafish embryo development (see Fig. 3).

In order to validate our algorithm and measure the accuracy of our implementation, we generated one synthetic 2D dataset that mimics a natural nucleus cell image (see Fig. 2(a)). In Fig. 2(b) demonstrates the effectiveness of our method to extract the synthetic cell. The estimated parameters are then perfectly recovered using our intensity profile fitting procedure.

We also validate our method on a single cell extracted from a larger data-set (see Fig. 2(c)). In Fig. 2(d), we show the resulting segmentation with our methods. Note that, with few iterations the level-set converges and the functional in Eq. 5 is minimized.

Finally, we tested our method on a real data set containing many cells (see Fig. 3). In this case, the initial level-set is provided by one biologist, expert with these data. We ran our algorithm and show the results in Fig. 3.

5. CONCLUSION

In this paper, we present an approximation of the intensity profile for nucleus of cell in microscopic images. This model is, then, integrated in a variational level-set formulation to segment one, or multiple, nuclei with the multiphase extension. We illustrate the effectiveness of our method on numerical simulation, as well as real data from zebrafish embryo images.

In future, we plan to validate our method and compare results of segmentation with hand-made segmentation from biologists. Then, this work will be used in biological studies on zebrafish development to automatically segment and track nuclei during the development of the embryo.

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Fig. 1. Image of the nucleus of one cell (left). Horizontal profile of the image intensity (middle). Vertical profile of the image intensity (right).

Fig. 2.

Synthetic image (a) of the nucleus of one cell with the initial implicit interface. Resulting of the segmentation using our method (b). Image of a nucleus with the initial implicit interface (c). Result of the segmentation using our method (d).

Fig. 3. Nucleii of a zebrafish embryo at an early stage are volume rendered (a). Middle z axis slice extracted from this volume (b). Given initialization for our level-set evolution method (c) and result of our multiphase segmentation method (d.)