CRF-DRIVEN MULTI-COMPARTMENT GEOMETRIC MODEL

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ABSTRACT

We present a hybrid framework for segmenting structures consisting of distinct inter-connected parts. We combine the robustness of Conditional Random Fields in appearance classification with the shape constraints of geometric models and the relative part topology constraints that multi-compartment modeling provides. We demonstrate the performance of our method in cell segmentation from fluorescent microscopic images, where the compartments of interest are the cell nucleus, cytoplasm, and the negative hypothesis (background). We compare our results with the most relevant model- and appearance-based segmentation methods.

Index Terms—Cell segmentation, multi-compartment segmentation, deformable models, conditional random fields

1. INTRODUCTION

An important task in microscopic imaging is to transform images into a numerically symbolic form for better representation, evaluation, content-based mining, as well as quantification of spatio-temporal interactions among the target structures. Segmentation of individual cells in a culture is part of our study, namely the assessment of the angiogenic effect of growth factor-delivery, as observed in cell proliferation [8]. In this study, we aim at enumerating cells and estimating the dominant orientation of the culture as denoted by the cell shapes and their spatial concentration.

Cell segmentation in microscopic images has been studied extensively in the literature. Some of the existing approaches focus on segmenting cells as a whole [12, 9, 1, 7, 5], while there have also been approaches that consider cells as multi-compartment structures [13, 6, 10, 3]. Segmenting cells as single-compartment objects is in general more straightforward, but not necessarily easier, depending on the data. In [12], the objective is formulated as a Conditional Random Field (CRF)-based classification problem. This work utilizes the information between neighboring spectral fields to achieve good segmentation results. Sertel et al. in [9] convert the RGB image into a unitone image using principle component analysis, and model the single-channel appearance with a bimodal Gaussian mixture, which provides the cell likelihood. In [5], Wang et al. detect proteins (dots) in cell nuclei and membranes (edges), with Mexican hat wavelets, which are used in an Adaboost classifier.

Multi-compartment cell segmentation essentially provides a solution that is constrained by the relative topology of the compartments. In [13], Yu et al. use two different level-sets to capture two cell parts. From color images, they first minimize the Mumford-Shah functional by evolving one level-set on the blue channel that indicates nucleus (Fig. 1). They assign zero intensity to the nucleus region in the green channel (indicating the rest of the cell) to match the intensity of the background. Then, they evolve the second level-set from the nucleus boundaries outwards to capture the cell membrane. In [6], a hierarchical Markov Random Field (MRF) is presented for multi-label segmentation. In our previous work [3], we used the multi-compartment shape representation of [2] in a deformable model, driven by the class assignments of a support vector machine. Finally, in [10], a multi-phase level set approach is presented, where the intra-cellular topology constraints are satisfied by introducing ribbon force fields in the image domain. This work differs from our method in the following. (a) In [10], it is assumed that the distance between the inner and outer boundaries of the ribbon is close to uniform and the range is a priori known; this usually cannot capture a highly varying ribbon thickness (see cytoplasm in Fig. 1). In our method we do not impose such constraints; we only assume that nucleus must be surrounded by cytoplasm. (b) Our method does not assume homogeneity within the distinct compartments, and we capture the intensity (color) variations with a locally adaptive CRF. (c) Our framework essentially provides a way of updating the probability field that drives the multi-compartment model evolution. (d) In [10], 2m level set functions are required for m ribbons (targets). In our work we use one distance function per compartment label, independently from the number
of part-defined targets. That is, extending the framework we present here to capture more than one cell, we would still use three distance functions (nucleus, cytoplasm, background).

To summarize, the solution we present here (i) provides local shape smoothness for the cell compartments, (ii) incorporates topology constraints among compartments, (iii) handles spurious observations, i.e., edges and intensity ambiguities, and (iv) uses shape priors to assist nuclei segmentation and consequently better constrain the solution for cytoplasm (where intensity inhomogeneities are more likely to appear). The integration of the CRF formulation in our objective provides increased accuracy, compared to [3], while our framework can be used in other general segmentation problems, when relative part topology (e.g., adjacency) is an inherent property of the data.

2. METHODOLOGY

Our objective is formulated as a shape and topology constrained geometric model driven by image likelihood fields. We use the blue and green chromatic components of the color image separately, to simultaneously capture the two regions of interest, namely the cell nucleus and cytoplasm. We obtain a topology initialization for our method with labeled seeds of interest, namely the cell nucleus and cytoplasm. We use the blue and green chromatic components of the color image separately, to simultaneously capture the two regions of interest, namely the cell nucleus and cytoplasm. We observe that the nucleus has a boundary that approximates an ellipse: this is a shape constraint we use in our framework, as the error of the least squares fitting of an ellipse to the nucleus boundary.

$$\varepsilon_s = \left( R_N \cap R_{\text{ellipse}} \right) / \left( R_N \cup R_{\text{ellipse}} \right),$$

with $R_N$ and $R_{\text{ellipse}}$ denoting the regions enclosed by the nucleus boundary and the ellipse, respectively.

The terms $J_{NB}$ and $J_{NC}$ quantify the areas of the joint parts of nucleus-background and nucleus-cytoplasm. Using the definitions in [2], let $M_{NB}$ and $M_{NC}$ denote the boundaries of $R_N \cup R_B$ and $R_N \cup R_C$, respectively. Also, let $m_{NB}$ and $m_{NC}$ denote the shared boundaries between nucleus-background and nucleus-cytoplasm, respectively. Then,

$$J_{NB} = \left\{ x \in R_N \cup R_B \mid \min_{y \in m_{NB}} ||x - y|| < \min_{z \in m_{NB}} ||x - z|| \right\},$$

$$J_{NC} = \left\{ x \in R_N \cup R_C \mid \min_{y \in m_{NC}} ||x - y|| < \min_{z \in m_{NC}} ||x - z|| \right\}.$$

During the minimization of the energy in eq. (3), the ratio $\int_{\Omega} J_{NB}(x) dx / \int_{\Omega} J_{NC}(x) dx$ forces evolution towards a configuration $\varphi$, such that (i) the nucleus does not share any boundary with the background, and (ii) the shared boundary between nucleus and cytoplasm is maximized. However, when the segmented cytoplasm regions are far from the nucleus, it is $\int_{\Omega} J_{NC}(x) dx = 0$. Therefore, to enforce the desired topology during the evolution of the three distance functions, in eq. (3) we introduce the factor $\kappa$, which regulates the
direction in which cytoplasm regions evolve towards the nucleus, as shown in Fig. 3. Specifically, let \( d(N, C) \) be the minimum distance between the regions \( R_N \) and \( R_C \). The locations \( x \) in \( \Omega \) between or inside the two regions minimize
\[
\alpha(x) = ||\varphi_N(x) + \varphi_C(x) - d(N, C)||, \quad \forall x \in \Omega
\]  
(7)
We define the functional,
\[
\kappa(x) = 1 - \mathcal{H}[\varphi_B(x)]e^{-\alpha(x)}, \quad \forall x \in \Omega
\]  
(8)
where \( \mathcal{H}[\varphi_B(x)] \) denotes the Heaviside function of \( \varphi_B \), i.e., \( \mathcal{H}[\varphi_B(x)] = 1 \Leftrightarrow x \in R_B \) and \( \mathcal{H}[\varphi_B(x)] = 0 \Leftrightarrow x \in \Omega \setminus B \), and is used to exclude the locations inside the nucleus and cytoplasm. When the two regions evolve towards each other, \( \int \kappa(x)dx \) increases, and the energy of eq. (3) decreases.

**External energy.** Let \( I_g(x) \) and \( I_b(x) \) be the intensities of the green and blue chromatic components at a pixel location \( x \). Also, let \( L = \{l(x)\}_{x \in \Omega} \) be the set of pixel labels: \( \{l(x) = 3, \forall x \in R_N \cup M_N\}, \{l(x) = 2, \forall x \in R_C \cup M_C\}, \{l(x) = 1, \forall x \in R_B \cup M_B\} \). For notation simplicity we will use \( \{N, C, B\} \) to denote the three labeled regions. To drive our objective in eq. (2), for each site on the image plane (here we use pixels) we calculate the conditional probabilities \( P(x \in \{N, C, B\}|I(x)) \), where \( I(x) = \{I_g(x), I_b(x)\} \). We use a Conditional Random Field to derive the corresponding image probability map, which provides the marginal distribution of labels at each pixel location \( x \) in \( \Omega \). Let \( E \) be the set of edges between neighboring nodes corresponding to pixel labels. Also, let \( d_{ij} = \|I(x_i) - I(x_j)\| \) be the feature vector that corresponds to the pairwise potential of two neighboring pixels \( i, j \) at locations \( x_i \) and \( x_j \), respectively, modeled by the edge \( (i, j) \in E \), \( i \neq j \). Then, the external energy of eq. (2) is defined as,
\[
E_{ext}(\varphi) = -\log P(L(\varphi)|\hat{I})
\]  
(9)
\[
P(L(\varphi)|\hat{I}) = \frac{1}{Z} \exp \left\{ \sum_{x_i \in \varphi^z} \sum_{x_j \in \varphi^{\varphi (i,j)} \in E} \left[ f(l(x_i), l(x_j)) \right] + g(l(x_i), l(x_j), d_{ij}) \right\}
\]  
(10)
where \( Z(\hat{I}) \) is the partition function, and \( \varphi^z \) denotes a narrow band of width \( 2w + 1 \) around the boundary of each region: \( |\varphi_N| \leq w, |\varphi_C| \leq w, \) and \( |\varphi_B| \leq w \). In our model, we allow for deformations only within this band, for faster convergence of the CRF. The singleton and pairwise potentials \( f \) and \( g \) have the same parameters throughout the entire image, and are defined as,
\[
\begin{align*}
\{ f(l(x_i), l(x_j)) = \log P(l(x_i)|\hat{I}(x_i)) \} \\
g(l(x_i), l(x_j), d_{ij}) = \exp \frac{\delta(l(x_i)-l(x_j))}{\sigma^2} \beta(d_{ij})
\end{align*}
\]  
(11)
We formulate the first potential using a support vector regression model with RBF kernel. For the Markovian property to be enforced, the optimal label configuration of a neighborhood is obtained with the pairwise maximization of \( g \), which penalizes neighboring pixels with different labels, controlled by \( \sigma^2 \) and the functional,
\[
\beta(d_{ij}) = \sqrt{[I_g(x_i) - I_g(x_j)]^2 + [I_b(x_i) - I_b(x_j)]^2},
\]  
(12)
which is the appearance difference between the two pixels.

**Implementation in steps:** (i) We initialize the set \( \varphi \) using intensity clustering of \( I_g \) and \( I_b \). (ii) We train the CRF using the regions \( \varphi > 0 \) as samples, and create the image likelihood map; we solve the inference using the loopy belief propagation algorithm in [4]. (iii) We minimize the objective energy in eq. (2) in a band of width \( 2w + 1 \) around the zero level of \( \varphi \), for the initial segments, and given the high certainty that they are seeds inside the desired regions, the band extends from the zero level outwards (not including pixels in the seed interiors). (iv) We update the intensity samples for \( \{N, C, B\} \), given the new configuration of the set \( \varphi \), and re-calculate the CRF parameters. (v) We repeat steps (iii)-(iv).

3. RESULTS

We tested our method using 150 single-cell instances from 20 different cultures. In our experiments, on average, we successfully segmented > 97% of the nuclei, with < 2% (of nucleus area) false positive assignments, and > 90% of the cytoplasm pixels, with < 5% (of cytoplasm area) false positive assignments. Fig. 4 shows a comparison between our method and two other segmentation frameworks, namely a Conditional Random Field and the multi-phase Chan-Vese model [11]. An immediate observation is that all methods segment the nuclei relatively accurately, while cytoplasm is more challenging due to the ambiguities in the green chromatic component. The CRF was ‘optimally’ trained, using samples from the same image. Our contribution is that we constrain the solution for cytoplasm regions using topology information, i.e., using (i) well-segmented nucleus as reference, and (ii) the fact that cytoplasm surrounds nucleus and background surrounds cytoplasm.

The open parameters in our framework are \( \mu \) and \( \nu \) of eq. (3). Considering \( \mu + \nu = 1 \), we assign higher weight \( \mu \) to shape smoothness when the cell is anticipated to be more symmetric (cytoplasm extending more symmetrically around
We presented a model-based framework for multi-compartment cell segmentation. The deformable model-like evolution of the compartment shapes is driven by image probability maps, local shape constraints, and relative topology constraints. In each step of the evolution, we update the image likelihood, and therefore, we can capture local intensity variations in a step-wise fashion. We sacrifice efficiency for robustness, a trade-off that we aim to tackle in our future work.

4. CONCLUSIONS

We presented a model-based framework for multi-compartment cell segmentation. The deformable model-like evolution of the compartment shapes is driven by image probability maps, local shape constraints, and relative topology constraints. In each step of the evolution, we update the image likelihood, and therefore, we can capture local intensity variations in a step-wise fashion. We sacrifice efficiency for robustness, a trade-off that we aim to tackle in our future work.

5. REFERENCES


