Cascaded Segmentation of Grained Cell Tissue with Active Contour Models

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Abstract

Cell tissue in microscope images is often grained and its intensities do not well agree with Gaussian distribution assumptions widely used in many segmentation approaches. We present a new cascaded segmentation scheme for inhomogeneous cell tissue based on active contour models. Cell regions are iteratively expanded from initial nuclei regions applying a data-dependent number of optimization levels. Experimental results on a set of microscope images from a human hepatoma cell line prove high quality of the results with regard to the cell segmentation task and biomedical investigations.

1. Introduction

One important part of the cellular response to stress is the rapid adaption of the translation processes. Stress granules (SGs) are dense aggregations in the cytosol emerging during cellular stress conditions and are assumed to be essential for mRNA storage during stress [11]. Processing bodies (PBs) on the other hand are suggested to be the places of mRNA degradation in eucaryotic cells [5]. As some SGs transiently associate with PBs this suggests that RNAs can be redirected to PBs for decay. To scrutinize these hypotheses it is essential to monitor occurrence and distribution of PBs and SGs in individual cells.

In fluorescence microscopy this is achieved by labeling certain SG/PB-components with different fluorochromes coupled to specific antibodies. Additionally, it is essential to detect the boundary and the nucleus of each cell to assign the structures to a certain cell. This allows the quantification of alterations in number, size and localization of SGs/PBs due to cell manipulations, e.g. viral infections [10]. Cell boundaries can be labeled by either antibody stainings or commercial dyes binding to membranes. Both strategies are strongly restricted as the number of antibodies reliably used in one experiment is limited and dyes (e.g. HCS CellMask) stain not only the outer membrane but also intra-cellular parts. Hence in this work, we aim at detecting the cell boundary on existing fluorescent images without need of further labeling, where we use the fluorescence channel for PBs in the following.

For cell segmentation we apply active contour models as the basic segmentation technique. The main contribution of this paper is a new cascaded segmentation scheme for active contours which allows to cope with inhomogeneous non-Gaussian intensity distributions of target objects like the grained cell tissue in our application. The overall approach is similar to [3], however, our new cascaded segmentation scheme overcomes the need for an additional dye to explicitly label cell tissue. In cascaded segmentation we make use of the fact that intensity distributions follow a coarse spatial pattern as the local average intensity of the PB-channel decreases towards the cell boundary. Snakes are optimized in a cascaded fashion over several levels whereas contours from the former level are used for initialization. Results on a set of test images prove the high performance of the proposed method with regard to the cell segmentation task and also to biomedical evaluation.

2. Related Work

Several segmentation techniques have been adapted to the special needs of cell segmentation in fluorescent microscopy images as a means for subsequent biological analysis. A level-set based approach for segmentation and tracking of HeLa cells using various fluorescent labelings is described in [4]. For segmentation of cells the fitting term [1] is replaced with a Gaussian likelihood for the intensities with unknown variance. In the rare case of lumped cells, these are separated using the watershed transform and subsequent region merging. A similar problem is treated in [9]. While for tracking of cells a particle filter is employed, snakes are used as a post processing step based on the snake energy [7]...
where the external energy is derived from the gradient vector flow field. In the integrated approach [3] for cell area segmentation and sub-cellular particle detection, cell segmentation is based on labeling with HCS Cell-mask. Initial contours are heuristically extracted based on k-means clustering and subsequently adapted using snakes. The snake energy incorporates a region fitting and gradient-based data term, combined with a regularization term, a shape prior to enforce elongated cells and a coupling term for multiple cells.

In [2] cell segmentation is modelled as statistical inference based on a graphical model where a random variable is assigned to each pixel. Potential functions are defined from a DAPI channel representing DNA content and cell boundaries detected in a differential interference contrast image. Final segmentation is derived via belief propagation to estimate the joint likelihood.

3 Cell Segmentation

Active contour models have proven suitable for object segmentation as their energy functionals allow easy integration of contour- and region-related energy terms [12]. In our scenario the number of target cells per image is detected in the DAPI channel, hence we take advantage of the topology preserving characteristics of parametric active contours. Each target cell \( i \) is modeled by a parametric contour function \( c_i : [0, 1] \rightarrow \mathbb{R}^2 \).

3.1 Coupled Active Contours

To segment multiple cells in a single image the individual snakes are not allowed to overlap, as overlapping cells do not occur in the biomedical setup. We adopt the approach of [12] for integrated optimization of \( N \) snake contours with a common energy functional:

\[
E(c_1, \ldots, c_N) = \sum_{i=1}^{N} E_s(c_i) + \rho \sum_{i=1}^{N} \sum_{j=i+1}^{N} \int_{\Omega_{i,j}} 1 \, d\Omega \tag{1}
\]

\( E_s(c_i) \) denotes the individual energy of a single snake, the second term penalizes for each pair of snakes \( c_i, c_j \) the overlap \( \Omega_{i,j} \) of their interior regions.

In our scenario the main clue to segment cells is region intensity. Thus we adopt the region-based snake energy [1]. Intensities of the interior of each cell and the background are modeled by different constant intensities and quadratic error terms. This is approximately equivalent to a Gaussian model with means \( c_i^{in}, i = 1 \ldots N \), and \( c^{bg} \) and with variances \( \lambda_{in} \) and \( \lambda_{bg} \):

\[
E_r(c_i) = \lambda_{in} \int_{R_{in}(c_i)} (I(x, y) - c_i^{in})^2 \, d\Omega + \lambda_{bg} \int_{\Omega \setminus \bigcup_{i=1}^{N} R_{in}(c_i)} (I(x, y) - c^{bg})^2 \, d\Omega, \tag{2}
\]

where \( R_{in}(c_i) \) denotes the interior of snake \( c_i \). Variances \( \lambda_{in} \) and \( \lambda_{bg} \) are specified as parameters, and mean intensities are optimally estimated after each gradient descent step as the means of the corresponding regions. For the final energy of a single snake a regularisation term is added penalizing curvature:

\[
E_s(c_i) = E_r(c_i) + \frac{1}{2} \int_0^1 \beta || c_i''(s) ||^2 \, ds \tag{3}
\]

As the region intensity distributions of cells in our scenario do not agree well with the intensity model this homogeneity criterion is quite restrictive. Thus we propose to embed it in a new cascaded segmentation scheme.

3.2 Cascaded Segmentation Scheme

Rather than following a Gaussian distribution the pixel intensities of the cells in our application tend to decrease monotonically with increasing distance from the nucleus region (cf. Fig. 2). Here we propose to segment such cells in a cascaded fashion by sequentially adding new cell fractions to the cell area. Fig. 1 shows an overview of the approach.

The basic idea of our approach is to replace the single optimization level commonly used with snake techniques by an iterative procedure with data-dependent numbers of levels. The state to which a snake converges at the end of one optimization level \( l \) is the basis for the initialization of the subsequent optimization level \( l + 1 \). In detail, the resulting snake region from
level \( l \) is dilated by 10 pixels and its contour yields the initial snake contour of level \( l + 1 \) to segment the adjacent cell area of darker intensities. In addition the area already segmented as part of the cell during level \( l \) is masked and thus excluded from further computations. Subsequently, \( c_{\text{en}}^{l\text{n}} \) and \( c_{\text{bg}}^{l\text{b}} \) are re-estimated from this initialization and are most likely smaller than prior values due to the intensity structure of the cells. Consequently, initializing the optimization procedure with dilated snake contours drives the snakes to further expand towards the background.

One important ingredient for such an iterative expansion scheme is a proper termination criterion. The segmentation should stop as soon as pixels are enclosed that more likely belong to the background than to the cells. To this end we consider the standard deviation \( \sigma_{\text{en}}^{l\text{n}} \) of intensity values within areas added to a cell in level \( l \) and the standard deviation within the background area \( \sigma_{\text{bg}}^{l\text{b}} \). As cell tissue staining is coarse-grained compared to the homogeneous background, iterative segmentation for a single cell terminates if the standard deviation of the interior intensity values of the recently added cell area falls below a threshold \( \theta_{\text{b}} \):

\[
|\sigma_{\text{en}}^{l\text{n}} - \sigma_{\text{bg}}^{l\text{b}}| < \theta_{\text{b}}, \tag{4}
\]

In addition, region growth is analyzed and very small snake expansion between two levels \( l - 1 \) and \( l \), i.e.

\[
|1 - \text{area}(c_{\text{i},l-1})/\text{area}(c_{\text{i},l})| < \theta_{\text{a}}, \tag{5}
\]

also results in termination of the optimization for the snake in question. The overall cascaded segmentation terminates if all individual snakes have terminated according to one of both criteria or if a maximum of 9 levels is reached.

Cell segmentation is initialized with snake contours as extracted from cell nuclei regions segmented by Otsu thresholding of the DAPI nuclei channel and morphological post-processing. For the majority of cells the nucleus is detected correctly, however, sometimes nuclei of neighboring cells are merged. To separate these we apply a simple yet efficient procedure adapted from [8]. For each resulting connected component a cell is hypothesized and its contour yields an initial snake.

### 4. Experimental Results

For experimental evaluation of the new segmentation scheme 8 images from epifluorescence microscopy are used. Each image consists of three channels, containing fluorescently labeled nuclei, SGs and, PBs respectively. SGs are labeled by immunostaining of TIAR (a protein localized in SGs), while PBs are labeled by immunostaining of DCP1a (decapping enzyme localized in PBs). The nuclei are labeled by DAPI. For evaluation a manual cell labeling is available as ground truth for all images. Three of the images belong to a control sample, the remaining five are infected with a virus as mentioned in the introduction. One control and two virus images containing 47 cells in total were used as training dataset, i.e. for estimating values for \( \lambda_{\text{en}} \), \( \lambda_{\text{bg}} \) and \( \theta_{\text{b}} \) (Tab. 1). The other parameters were determined empirically. The remaining five images with 87 cells form the test dataset.

<table>
<thead>
<tr>
<th>Level ( l )</th>
<th>( \lambda_{\text{en}} )</th>
<th>( \lambda_{\text{bg}} )</th>
<th>( \rho )</th>
<th>( \beta )</th>
<th>( \theta_{\text{a}} )</th>
<th>( \theta_{\text{b}} )</th>
</tr>
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<td>1&amp;2</td>
<td>45</td>
<td>250</td>
<td>( 10^5 )</td>
<td>0.75</td>
<td>0.013+</td>
<td>100</td>
</tr>
<tr>
<td>( \geq 3 )</td>
<td></td>
<td></td>
<td></td>
<td>1.25</td>
<td>0.002 \cdot \text{l}</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Optimization parameters.**

In Fig. 3 (a) box plots of recall and precision of the cell area for all 87 cells of the test set are shown over a maximum number of 9 levels. As the cell boundary segmentation starts with the nuclei contours (Fig. 2, left) initially large fractions of the cells are missing yielding low recall values. During cascaded segmentation further parts of the cells are included (Fig. 2, right) resulting in an increase of the recall from 0.505 after the 1st level to 0.902. In contrast, the precision is initially high with 0.998 as almost no non-cell pixels are included, however over time decreases to a still very satisfying value of 0.865. In some cases low precision for single cells results from inclusion of other cells’ tissue, mainly at image boundaries where tissue is visible, but no corresponding nucleus exists. Note that 90\% of the snakes are optimized for at least 3 levels, while more than 85\% of the snakes terminate after a maximum of 5 levels.

From a biological point of view accurate cell boundary detection is required to correctly estimate number and size of SGs and PBs per cell. Both SGs and PBs are detected applying the scale-adaptive wavelet-based segmentation approach published in [6]. Some PB detection results are shown in Fig. 2. In Fig. 3 quantitative detection and evaluation results for the test dataset are shown. Fig. 3 (b) shows scatter plots of the number of SGs and PBs detected per cell in ground truth labeled vs. automatically segmented cells for the test set. Results after the first level are shown in blue, final results in red. Spearman correlation coefficients improve from 0.877 for SGs and 0.835 for PBs after the first level to very good 0.925 and 0.932 after termination of the cascaded approach, proving the high quality of segmentation.

In Fig. 3 (c) the area fractions of PBs and SGs for the 31 cells of the control set and the 56 cells of the virus-infected test cells are depicted. Area fraction is defined...
as the ratio between the area of a cell which is populated by structures (i.e., PBs or SGs) and complete cell area. The values clearly show that control cells (blue) contain a certain number of large PBs while with a few exceptions SGs remain absent. In contrast, upon viral transfection (red) SG-formation is induced in a portion of the cells, which shows the cells presumably as indeed transfected. This correlates with a reduced PB number and size. The results clearly outline that not every cell in the image undergoes such alterations and, thus, widely-used average quantification over complete images is biased and does not reflect the situation correctly.

5. Conclusion

The paper presents a new cascaded segmentation technique based on coupled active contours which is particularly well-suited to segment objects with non-homogeneous and non-Gaussian intensity distributions. The segmentation of target cells is iteratively expanded yielding very satisfying recall and precision of cell area on the test dataset. Also comparing the numbers of structures detected per cell with ground truth data shows the high quality of segmentation results. Summarizing, the proposed automated segmentation of fluorescent images shows as a valid basis for biological analysis and interpretation of cellular processes.

References