Published in final edited form as:

Int Conf Signal Process Proc. 2013 September 18; : 670–674. doi:10.1109/ICIP.2013.6738138.

Drosophila Eye Nuclei Segmentation Based on Graph Cut and Convex Shape Prior

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Abstract

The rapid advance in three-dimensional (3D) confocal imaging technologies is rapidly increasing the availability of 3D cellular images. However, the lack of robust automated methods for the extraction of cell or organelle shapes from the images is hindering researchers ability to take full advantage of the increase in experimental output. The lack of appropriate methods is particularly significant when the density of the features of interest is high, such as in the developing eye of the fruit fly. Here, we present a novel and efficient nuclei segmentation algorithm based on the combination of graph cut and convex shape prior. The main characteristic of the algorithm is that it segments nuclei foreground using a graph cut algorithm and splits overlapping or touching cell nuclei by simple convex and concavity analysis, using a convex shape assumption for nuclei contour. We evaluate the performance of our method by applying it to a library of publicly-available two-dimensional (2D) images that were hand-labeled by experts. Our algorithm yields a substantial quantitative improvement over other methods for this benchmark. For example, our method achieves a decrease of 3.2 in the Hausdorff distance and an decrease of 1.8 per slice in the merged nuclei error.

Index Terms

drosophila eye; fluorescence microscopy image; nuclei segmentation; graph cut; convex and concavity analysis

1. Introduction

The importance for biomedical applications of identifying cells, nuclei and other organelles in microscope images has led to the development of image analysis approaches able to

automatically segment such features from images. Current methods for cell, nuclei, and other organelle segmentation include watershed algorithms [1, 2], supervised machine learning methods [3], a level set active contour model [4], multiscale analysis [5], dynamic programming-based methods [6, 7], graph-cut methods [8, 9, 10], and Markov random fields [11]. Among these methods, graph cut methods offer the important advantage of yielding globally optimal solutions. However, graph cut algorithms requires effective initialization and are unable to separate touching cells or nuclei [3].

In this paper, we present a fully automated method, based on graph cut with convex nuclei shape prior, for segmentation touching nuclei that, we believe, addresses most of challenges associated with the segmentation of fluorescence microscopy images. Our method's novelty is grounded on two characteristics. First, we propose a new convex-concavity analysis method and an optimal splitting path searching method that are simple and efficient. Second, we combine our proposed convex-concavity analysis for nuclei segmentation with a graph cut algorithm with easy initialization.

The rest of the paper is organized as follows. We present the graph cut for nuclei foreground segmentation in Section 2, and a detailed description of our convex-concavity analysis in Section 3. In Section 4, we report our segmentation results of drosophila eye nuclei in fluorescence microscopy images and demonstrates the improved performance of our algorithm compared to other methods evaluated on a publicly-available database. Finally, we end the paper with some concluding remarks (Section 5).

2. Graph Cut Segmentation for Nuclei Foreground

The first step in nuclei segmentation is to separate the foreground pixels in the nucleichannel $I_N(x, y)$ from the background pixels. Therefore, we will use graph cut with two terminals to segment the nuclei foreground. Consider an arbitrary set of data elements (pixels or voxels) $\mathcal{P}$ and some neighborhood system represented by a set $\mathcal{N}$ of all (unordered) pairs $\{p, q\}$ of neighboring elements in $\mathcal{P}$. Let $A = (A_1, \dots, A_p, \dots, A_{|\mathcal{P}|})$ be a binary vector whose components A_p can be either “obj” or “bkg” (abbreviations of “object” and “background”). Vector A defines a segmentation. The graph cut algorithm minimizes the following energy function [8]

$$E(A) = \lambda \cdot R(A) + B(A) \quad (1)$$

where

$$R(A) = \sum_{p \in \mathcal{P}} R_p(A_p) \quad (\text{regional term}) \quad (2)$$

$$B(A) = \sum_{\{p, q\} \in \mathcal{N}} B_{p, q} \cdot \delta_{A_p \neq A_q} \quad (\text{boundary term}) \quad (3)$$

and

$$\delta_{A_p \neq A_q} = \begin{cases} 1 & \text{if } A_p \neq A_q \\ 0 & \text{if } A_p = A_q. \end{cases}$$

The coefficient $\lambda = 0$ in (1) specifies the relative importance of the region properties term $R(A)$ versus the boundary properties term $B(A)$. The regional term $R(A)$ assumes that the individual penalties for assigning pixel p to “object” and “background”, correspondingly $R_p(\text{“obj”})$ and $R_p(\text{“bkg”})$, are given. For example, $R_p(\cdot)$ may reflect on how the intensity of pixel p fits into given intensity models (e.g., histograms) of the object and background

$$R_p(\text{“obj”}) = -\ln \Pr(I_p | \text{“obj”}) \quad (4)$$

$$R_p(\text{“bkg”}) = -\ln \Pr(I_p | \text{“bkg”}) \quad (5)$$

In this paper we use the mixture of two Poisson distributions to model the pixel intensity distribution $\Pr(I_p | \text{“obj”})$ and $\Pr(I_p | \text{“bkg”})$ in fluorescence microscopic images [9]. This model can be simply estimated using an image histogram. We omit the details of model estimation which can be found in [9]. There is no parameter to be tuned for initialization of the term $R(A)$.

The term $B(A)$ comprises the “boundary” properties of segmentation A . Often, it is sufficient to set the boundary penalties from a simple function like [8]

$$B_{p,q} \propto \exp\left(-\frac{(I_p - I_q)^2}{2\delta^2}\right) \cdot \frac{1}{\text{dist}(p, q)}. \quad (6)$$

This function provides a large penalty for discontinuities between pixels of similar intensities when $|I_p - I_q| < \delta$. However, if pixels are very different, i.e., $|I_p - I_q| > \delta$, then the penalty is small. Here we want to segment the nuclei foreground. The value of δ can be set to a relatively large value (30 in this paper) without caring about nuclei clustering. Therefore, the graph cut algorithm can be initialized easily by setting λ and δ equal to 1 and 30, respectively. Fig. 1 shows the drosophila eye nuclei segmentation result with multiple nuclei clusters using the graph cut algorithm described above. In the image on the left the boundary contour of segmented image overlaying with original gray scale image is shown, while in the image on the right the binary image segmented by graph cut without splitting process is shown. The largest cluster is highlighted in red color. In our confocal microscopic images high nuclei density challenges the segmentation algorithm. To address this kind of nuclei clustering problem, a splitting algorithm for nuclei clumps is given in the following section.

3. Splitting of Touching Nuclei

The recently segmented nuclei are not properly separated as shown in Fig 1, and thus they need to be separated. For this a new simple convex-concavity analysis method is proposed here with the assumption that each nucleus has convex shape.

Let R be a simply connected region bounded by a rectifiable simple closed curve C (Fig. 2). The convex hull of R , denoted by $\hat{R}$ is simply connected and its boundary consists of a finite sequence of curves $C_1, C_2, C_3, \dots$. Each $C_j, j = 1, 2, 3, \dots$, is either an arc or a chord of C . Each chord denoted by $K_i, i = 1, 2, 3, \dots$, is a line of support of C . B_i is the curve with the same end-points as K_i but belongs to the boundary curve C . The point on curve B_i with the longest distance d_i to corresponding chord K_i is denoted by P_i . The point P is called the steepest concave point (SCP) if

$$P = \arg \max_{P_i} d_i. \quad (7)$$

The subscript index of steepest concave point P is denoted by t . In Fig. 2, the steepest concave point is P_2 and the subscript index of the steepest concave point is $t = 2$. Point among all points on boundary curves $\{B_i, i \neq t\}$ is defined as the nearest boundary point (NBP) such that

$$NBP = \arg \min_{P \in \{B_i, i \neq t\}} D(P_t, P), \quad (8)$$

where $D(P_t, P)$ is the distance between the steepest concave point P_t and the boundary point P lying on boundary curves $\{B_i, i \neq t\}$. It should be noted that point P in equation 8 should not lie on the boundary curve B_t where the steepest concave point P_t resides. The line connecting point SCP and point NBP is an optimal splitting path, as shown in Fig. 2 in blue color. If the largest distance d_t corresponding to the steepest point P_t is less than a distance threshold T_d (in this paper $T_d = 5$ for 40X lens in a confocal microscope), then the task of splitting connected component R is completed. Otherwise, the connected component R is split into two parts R_1, R_2 along the optimal separating path. For each split part, repeat the above separating process until distance d_t is less than a threshold T_d .

Our touching nuclei splitting algorithm based on our convex-concavity analysis is as follows:

Algorithm 1 Nuclei Segmentation

- 1 Using graph cut to segment nuclei foreground and obtain binary image.
- 2 Collect all connected components from segmented foreground into list L .
- 3 For each connected component R in list L , find the steepest concave point P_t according to equation 7.
- 4 If corresponding d_t is less than threshold T_d , delete this connected component R from list L and return to step 3. Otherwise, go to step 5.
- 5 Find the corresponding nearest boundary point NBP according to equation 8.

- 6 Split connected component R into two parts R_1 and R_2 along the optimal separating line segment connecting point P_i and NBP . Add the split parts R_1 and R_2 back to the list L .
- 7 Iterate Steps 3, 4, 5, 6 until list L is empty.

The effect of using this convexity and concavity analysis based splitting method is illustrated in Fig. 3 which shows an example of splitting one large connected component we encounter in our segmentation result. One and two line segments (in red color) found by our splitting method are shown in Figs. 3(b) and (c), respectively, where red circles denote the vertices of the convex hulls of connected components. Fig. 3 shows that our convexity and concavity analysis based method can separate nuclei clustered together.

4. Experimental Results

We applied our approach to fluorescence microscopic images of a drosophila developing eye. The total number of slices is 78 and each has size 1024×1024 pixels. These images only scan half of the developing drosophila eye and are taken with a 40X lens in a confocal microscope. Cell nuclei are labeled in red, and the protein of interest, which is called YAN, is labeled in green; yellow is where the two colors coexist. Our aim is to measure the abundance of the protein of interest (green channel). The idea is to use the red channel as a sort of a map, to be able to unambiguously identify the nuclei positions and calculate fluorescence intensity of the green channel inside the nuclei. For that we need to segment the nuclei first.

The segmented results for the third whole slice (s3) and ninth whole slice (s9) are shown in Fig.4 to illustrate the effect of our segmentation algorithm proposed in this paper in dealing with the high nuclei density problem. Figs. 4(a), (b) are showing contours (blue curves) and centers (red dots) of segmented nuclei overlaying on the original red channel images, respectively. It can be seen from this figure that our method proposed here can separate tightly touching nuclei within high nuclei density images.

Next we provide a quantitative evaluation of the performance of our proposed method in this paper based on the 2D nuclei segmentation benchmark from [12]. We use the same measures for the qualitative evaluation, such as merge, split, spurious and missing, and those for quantitative evaluation like Rand Index (RI) and Hausdorff distance. Fig. 5 shows our segmented result on the “difficult” image (U2OS data set) suggested in [12]. Figs. 5(a), (b) are original gray scale image and ground truth, respectively. Our segmented and split result is shown in Fig. 5(c). Fig. 5(d) shows the comparison of contours of nuclei found by hand (green contours) and our algorithm (red contours). It is demonstrated that our algorithm in this paper can perfectly deal with the difficult segmentation problem rising from many clustered nuclei.

In [12], several published algorithms have been implemented and tested with the data set U2OS using the objective metrics of Table 1. To compare our algorithm in this paper with these algorithms in [12], we run it on the same benchmark data set U2OS and list the values of several metrics in Table 1. The values of different metrics for all algorithms except ours are directly from [12]. The results in Table 1 show our superior performance in most categories. In particular, due to the global optimality property of graph cut and convex shape

prior, our method provides significant quantitative improvement (that is 3.2 Hausdorff distance decrease and 1.8 decrease in the merged nuclei error per slice over the best method (referred to as “Merging Algorithm”) in [12].

5. Conclusion

In this paper, we reported on new method that combines graph cut and a convex shape prior for the extraction of features from confocal microscope images. We applied our method to a publicly-available, hand-curated, 2D benchmark and demonstrated that our method easily outperforms all of the segmentation methods implemented in [12]. We also applied our method to a slices from a 3D stack for nuclei segmentation of drosophila eye tissue obtained with fluorescent-microscopy images and verified that it yields excellent agreement with expert-segmentation. Our results suggest that our method could be of great value to experimental biologists working with confocal microscope images.

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Fig. 1. Nuclei foreground segmentation using graph cut with multiple nuclei clusters: left: boundary contour of segmented image overlaying with original gray scale image; right: binary image segmented by graph cut without splitting process. The largest connected component is highlighted in red color in the right image.

Fig. 2.

A connected component R and its convex hull. C_2 , C_4 and C_6 are arcs of the boundary contour C enclosing R , while C_1 , C_3 , C_5 are chords of C denoted respectively by K_1 , K_2 , K_3 . B_1 , B_2 , B_3 are boundary curves with the same ending points as chords K_1 , K_2 , K_3 , respectively. P_1 , P_2 , P_3 are the points on the boundary curves B_1 , B_2 , B_3 which have the largest distances d_1 , d_2 , d_3 from all points on B_1 , B_2 , B_3 to chords K_1 , K_2 , K_3 , respectively. P_2 is called the steepest concave point (SCP). Optimal splitting path is the line segment in blue color connecting points SCP and the nearest boundary point (NBP).

Fig. 3.

Illustrating key steps of the proposed nuclei splitting method. (a) one large connected component. (b) The first optimal splitting path (highlighted by red color) found by the first iteration in our splitting method. Red circles indicate the vertices of the convex hull of the connected component. (c) Two optimal splitting paths (highlighted by red color) found by the second iteration in our splitting method. Red circles indicate the vertices of convex hulls of the connected components (two connected components indicated by green convex hulls).

Fig. 4. Sample segmentation results of two whole slices (s3 and s9) with high density of nuclei. (a), (b) contours (blue curves) and centers (red dots) of segmented nuclei overlaying on original red channel images of s3 and s9, respectively.

Fig. 5. Comparison of our segmented result with hand segmented result on the “difficult” example given in [12]. (a) original gray scale example; (b) ground truth (red curves);(c) segmented result with our algorithm; (d) green contours (golden standard) and red contours (our algorithm) of nuclei.

Table 1
Comparison of segmentation algorithms. The statistical values for all methods except ours are directly from [12]

Algorithm	RI	Jl	Hausdorff	NSD ($\times 10$)	Split	Merged	Spurious	Missing
AS Manual	95%	2.4	9.7	0.5	1.6	1.0	0.8	2.2
RC Threshold	92%	2.2	34.8	1.2	1.1	2.4	0.3	5.5
Otsu Threshold	92%	2.2	34.9	1.2	1.1	2.4	0.3	5.6
Mean Threshold	96%	2.2	26.5	1.0	1.3	3.4	0.9	3.6
Watershed (direct)	91%	1.9	34.9	3.6	13.8	1.2	2.0	3.0
Watershed (gradient)	90%	1.8	34.6	3.0	7.7	2.0	2.0	2.9
Active Masks	87%	2.1	148.3	5.5	10.5	2.1	0.4	10.8
Merging Algorithm	96%	2.2	12.9	0.7	1.8	2.1	1.0	3.3
Our Algorithm	96%	2.5	9.7	0.6	1.4	0.3	0.9	3.3