Advanced Segmentation of Nuclei Using Level Set and Watershed Methods

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Abstract

Abnormalities in number and location of brain cells are hallmarks of both developmental and degenerative diseases. Our aim is to accurately quantify differences in cells density and distribution in large tissue volumes between normal and pathological brain tissue. First step to accomplish this goal involves accurate segmentation of neurons. Here we describe an automated approach to segmentation of the brain cell nuclei of embryonic mouse brain in imaged by laser scanning microscopy (LSM). The nuclei are first segmented using the level set (LS) method. Because LS tends to merge clustered cells resulting in under-segmentation, it is followed by the watershed segmentation (WS) that in turn tends to produce over-segmentation. Therefore, we developed the segmentation correction rules that are based on topological properties of the nuclei of interest to further refine the segmentation results. The obtained segmentation results are compared to manual segmentation performed by an expert. The accuracy of our automated method is estimated at 97%. We conclude that our approach might provide a useful tool for stereology of LSM brain tissue images.

Introduction

Abnormalities in the number of cells and their location are important hallmarks of both developmental and degenerative neurological diseases including forms of epilepsy and mental retardation, as well as Parkinson's disease and Alzheimer's disease. Each cell contains a single nucleus. Therefore, the number and location of nuclei provide quantitative information about the number and spatial arrangement of cells in the normal and diseased brain.

The accurate segmentation forms the framework for the number and location of nuclei, and a much deeper understanding of neuronal architecture and diseases. A number of image analysis methods have been developed for nucleus segmentation [1-3]. For example, a statistical model, based on Compact Hough Transform, likelihood function and global grey-level histogram information, has been developed for cell nuclei segmentation in [1]. Nedzved [2] designed the morphological operators to segment cells in images with sparse density. A watershed method with recursive tree-based algorithm is presented in [3] to segment nuclei in the rat hippocampus. However, over-segmentation and under-segmentation are still the main problems in the performance results, which affect the further image analysis task, e.g. cell detection and localization. Meanwhile, most of the above methods are based on two-dimensional intensity information without considering the effect of three-dimensional overlapping of cells.

In this paper, we combine advancements in 3D fluorescence-based microscopy with emerging image segmentation approaches to develop a fully automated method for both representation and quantification of neuronal cytoarchitecture. The level set segmentation has a number of practical and theoretical advantages over conventional segmentation methods. However, it is sensitive to the initial placement of the contour and tends to merge clustered cells, while watershed segmentation forms connected paths, giving continuous boundaries between regions. Therefore, once level set segmentation is done, the watershed segmentation method is applied to the binary image that the level sets return. Though most of the nuclei are distinctly separated after the above steps, some of them are still under-segmented or over-segmented, especially in the case of the overlapping nuclei. To alleviate this situation we develop segmentation correction methods and apply them to the segmented images.

Segmentation of Neuronal Nuclei in the Synthesized Image Volume

Nuclei segmentation methods can be divided to two classes: region-based and boundary-based. Region-based segmentation focuses on whether a voxel belongs to an object or not, whereas the boundary-based approach tries to find the voxels on the boundary of an object. Our paper is based on the level set and watershed segmentation.

Implementation of level set segmentation

Level set segmentation is a method for tracking contours and surfaces. The main idea of the level set method is to represent a curve as the zero level set of a higher dimensional function; the motion of the curve is embedded within the motion of the higher dimensional surface. The main advantages of using the level set method is that arbitrarily complex shapes can be modeled and topological changes such as merging and splitting are handled implicitly.

Level sets segmentation uses image-based features such as mean intensity, gradients and edges to design differential equations. Many different implementations and variants of this basic concept have been published. Due to its ability to handle topological changes naturally, we apply the C-V level set method in this project [4].

Implementation of watershed segmentation

Watershed segmentation is a powerful and efficient technique in region-based segmentation. Watershed segmentation is based on developing a three-dimensional image with two spatial coordinates versus gray levels. More details about watershed segmentation can be found in [5-7].

The watershed algorithm is summarized below:

1) Create a Euclidean distance map of the binary image.

2) Create a map of "Ultimate Points" by eroding each object until only one pixel remains. One object can have multiple "ultimate points", especially in the case of overlapping nuclei.

3) Dilate the image from each of these points until a) it hits the original object boundary, or b) it hits the dilation of another object. In case b, a one-pixel-wide boundary of background color separates the colliding dilating objects.

Development and Implementation of Segmentation Correction Rules

The approach to improve the accuracy of segmentation is to utilize the spatial information to refine the segmentation results in 3D. As we know, there are two different types of information available in the image: a) the intensity values of the voxels and b) their spatial interdependency. Employing only intensity values in segmentation results in increased sensitivity to the noise present in the images. It is necessary to consider information about the neighboring voxels in adjacent optical planes to improve the segmentation.

The centroid of a nucleus i in the t plane (C_i^t) is defined as a central point of its skeleton in 3D. The segmentation correction method is described below [8]:

1) Collect the centroids' information, including their location, the distances between each pair of centroids, and the nucleus radius R_{ci};

2) For a specific centroid C_i^t in the tth plane, locate the nearest centroid C_i^{t+1} in the $(t+1)^{th}$ plane. If the distance between them is smaller than αRci, where α is a constant, the corresponding centroids in t^{th} and $(t+1)^{th}$ slices belong to the same nucleus then move to step 7);

3) If the distance is more than αR_{ci}, then these two centroids do not belong to the same nucleus or the segmentation is incorrect;

4) Let D_i^t be the minimum distance between C_i^t and other centroids in the same plane, and D_i^{t+1} be the minimum distance between C_i^{t+1} and other centroids in the $(t+1)^{th}$ plane. If D_i^t is larger than D_i^{t+1}, which means that one more centroid corresponding to C_i^t may appear in $(t+1)^{th}$ plane, we name this situation '1->2' to state that two or more centroids are found instead of just one. Fig. 1 (a, d, b, and e). Similarly, if D_i^t is smaller than D_i^{t+1}, it belongs to '2->1' situation, Fig. 1 (b, e, c, and f);

5) Repeat step 2) in the $(t+1)^{th}$ plane and $(t+2)^{th}$ plane, and if C_i^{t+1} and C_i^{t+2} belongs to the same nucleus, move to step 7); otherwise repeat step 3) and 4) for C_i^{t+1} and C_i^{t+2};

The selected decision rules are listed in Table 1. Then we decide which segmentation is incorrect segmentation and correct the segmentation results.

Move to the C_{i+1}^t centroid in the tth plane;

Repeat the steps 1)– 7) until the last plane is reached.

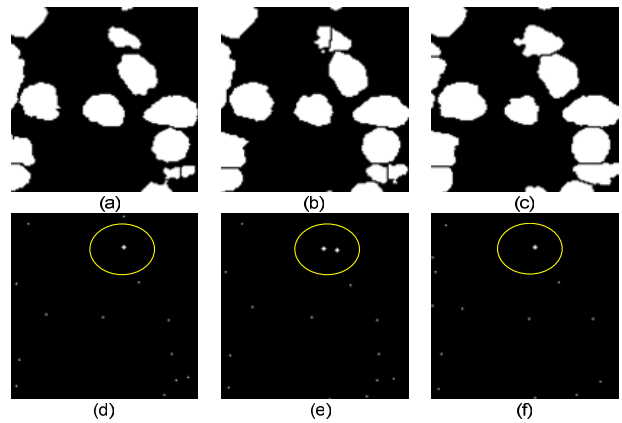

***Figure 1**. An over-segmentation example based on slice 17th, 18th, and 19th. (a) Plane 17th after level set and watershed segmentation. (b) Plane 18th after level set and watershed segmentation. (c) Plane 19th after level set and watershed (d) Estimated centroids of the nuclei in (a). (e) Estimated centroids of the nuclei in (b). (f) Estimated centroids of the nuclei in (d)segmentation. (d) Centroids of plane 17th. (e) Centroids of plane 18th. (f) Centroids of plane 19th.*

Table 1 shows the decision rules used to correct over-segmentation or under-segmentation with the spatial information between planes. The tth, $(t+1)^{th}$ and $(t+2)^{th}$ denote the number of nuclei in a specific region in the tth plane, the $(t+1)^{th}$ plane and the $(t+2)^{th}$ plane, respectively. For example, if there is one nuclei in that region in the tth and $(t+2)^{th}$ plane, but two nuclei in the $(t+1)^{th}$ plane, we decide that there is a over-segmentation in the $(t+1)^{th}$ plane. An illustration of decision rules is shown in Fig.2.

Table 1 Heuristic decision rules for over-segmentation or under-segmentation

t^{th} $(t+1)^{th}$ $(t+2)^{th}$	Decisions
1 → 2 → 2	Under-segmentation in t^{th}
2 → 1 → 1	Over-segmentation in t^{th}
1 → 2 → 1	Over-segmentation in $(t+1)^{th}$
2 → 1 → 2	Under-segmentation in $(t+1)^{th}$

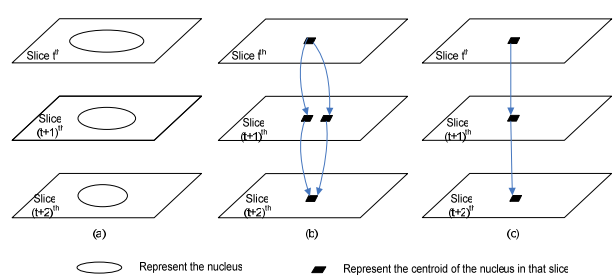

***Figure 2**. An over-segmentation decision example. (a) Nucleus in slice tth, (t+1)th, and (t+2)th. (b) Centroid for the nucleus in each slice, an over-segmentation is found in the (t+1)th slice. (c) Corrected centroid in slice (t+1)th.*

Experimental Results

Our segmentation correction method has been tested on LSM images obtained from an embryonic mouse brain and compared to centroids segmentation performed by an expert on the same images. The accuracy of segmentation performance is about 97% compared to the expert results. The initial results obtained using selected algorithms are shown in Fig. 3. In Fig. 3, an over-segmentation appears in plane 18 and is eliminated after correction using the spatial information from planes 17 and 19. We use $\alpha=0.5$ in all the experiments.

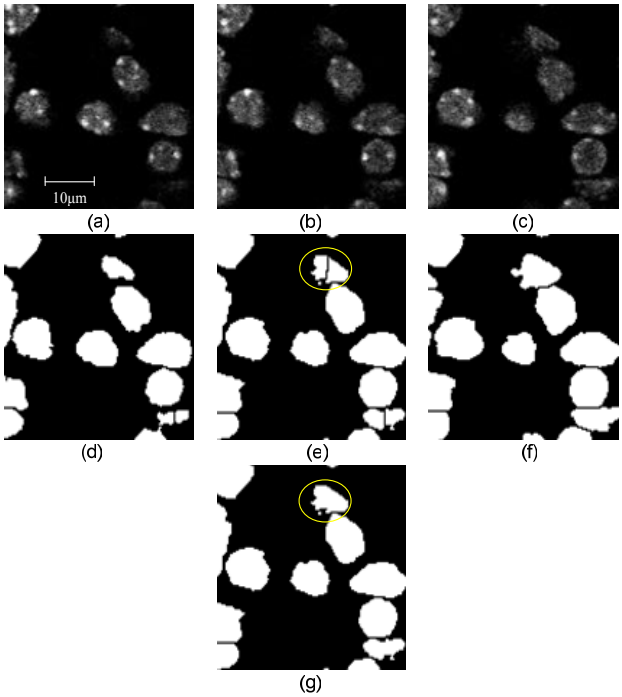

***Figure 3**. Example of segmentation correction. (a)-(c) Original laser scanning 2-photon microscopy images of brain tissue in planes 17 through 19. (d)-(f) Corresponding binary images (planes 17 through 19) with nuclei segmented using our algorithms. The circled nucleus in plane 18 is incorrectly segmented. (g) Correction of the segmentation in plane 18 using the decision rules and information from the adjacent planes.*

Conclusions

A sequence of algorithms including the level set and the watershed segmentation followed by segmentation correction rules has been successfully applied to accurately segment neuronal nuclei in the cell dense embryonic brain tissue volumes. Future research will focus on developing a set of largely heuristic rules for handling incompletely sampled data, for instance, to properly handle the nuclei on the boundary of the imaged volume.

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Author Biography

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