A 3D SELF-ADJUST REGION GROWING METHOD FOR AXON EXTRACTION

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ABSTRACT

Neuron axon analysis is an important means to investigate disease mechanisms and signaling pathways in neurobiology and often requires collecting a great amount of statistical information and phenomena. Automated extraction of axons in 3D microscopic images posts a key problem in the field of neuron axon analysis. To address tortuous axons in 3D volumes, a self-adjust region growing approach referring to surface modeling and self-adjustment which takes advantage of the nature of axon (e.g., continuity), is presented. Experimental results on axon volumes show that the proposed scheme provides a reliable solution to axon retrieving and overcomes several common drawbacks from other existing methods.

Index Terms— region growing, self-adjust, neuron axon

1. INTRODUCTION

Neurons are composed of dendrites, cell body, synapses and axons. As a specialized part of neuron, axon is crucial in all research that attempts to reconstruct connectivities of neurons. Hence, the research of axon structure acts an important role [1] in investigating nervous system.

The tortuous nature of the axons necessitates the reconstruction of axon trajectories through manual selection of 2D slices, a particularly time-consuming task that often requires plenty of neurobiologists' time. In order to process large volume of datasets and extract quantitative results, it is necessary to develop an automatic 3D axon detection algorithm. Rapid, automated cell axon segmentation and tracking would allow immediate visualization of the axon trajectory, thereby permitting assessment of cell axon modality directly. A variety of approaches have been proposed in the literature which can be broadly classified into two groups, 2D-based and 3D-based methods. 2D-based methods are often involved with detecting axons in individual slices of 3D datasets and synthesizing correspondence between slices. Because the tortuous nature of axons often makes the tracking through slices endure a lot of correction, 3D-based processing is considered to be a reasonable solution. A wide range of 3D-based techniques have been proposed, such as 3D mathematical morphology methods [1], 3D deformable model based methods [2], and 3D region growing. A profound review of tubular object extraction algorithms including many 3D-based methods can be found in [3]. However, in order to make use of the spatial continuity of axons other than the intensity contrast between axons and background, 3D region growing seems to be the most congruent method to extract axon trajectory.

3D region growing is a technique which begins with a seed location and attempts to extract a connected region which corresponds to a meaningful object from 3D volume. For the segmentation of tubular shapes and of vessels in particular, high level constrains have been introduced to achieve better performance. In [4], vessel direction and regional measure are used to control the evolution of region growing. In [5], region probability estimation and region classification are employed to express prior knowledge before segmentation. However, the pitfalls characterizing the cell axons is the touching problem which is a prevailing phenomenon resulted from multiple closed axons which cannot be detached correctly even by human. Thus, while these methods are adequate for segmentation of vessels which seldom wind together, they cannot truncate the wrongly grown part during the evolution of region growing.

Our work aims to develop an image segmentation methodology for automatically extracting the whole axon meshwork from 3D dataset acquired using confocal microscopy. Inspired by the evolution process associated with region growing, in which axon continuity, shape, and conformation are implicitly expressed, an algorithm for axon extraction is proposed. By modeling the evolution and introducing high level shape constraints and prior knowledge, a self-adjust region growing is obtained for extracting an axon from the 3D dataset, as described in

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Section II, and the results are presented in Section III. Finally, Section IV provides our conclusions.

2. PROPOSED METHOD

The presented method falls into the category of region growing approach as described previously. However, multiple techniques which introduce shape constrains are adopted to deal with the specific characteristics of axon segmentation and extraction.

During the evolution of region growing, every voxel which is in the neighborhood of the grown region is checked with homogeneity criterion. However, this decision-making process is only based on limited information such as the intensity of voxel itself and its neighborhood, so it is hard to introduce high-level prior knowledge to guide the region growing. Unlike other methods which try to detect and minimize the error at every generation using mechanisms like adaptive threshold [4], our method model and analyze previous generation's results to prevent error from spreading as shown in Fig. 1. In order to introduce shape constraints and self-adjust abilities, we describe the proposed region growing algorithm with basic concepts and definitions.

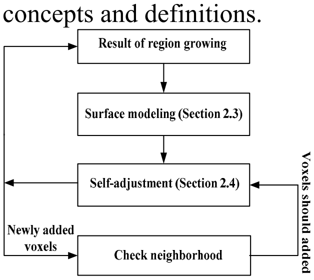


Fig. 1 Flow chart of the proposed approach.

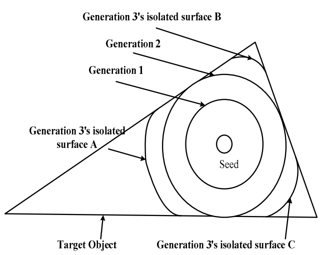


Fig. 2 Generation, surface, and isolated surface.

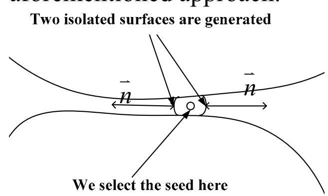
2.1 Basic concepts

2.1.1 Generation, surface and isolated surface

Generation is an important property of voxels added to the grown region which indicates the order of added pixels in region growing. The value of the seed point is set to 1 and the value increases while region growing. As a result, a voxel with a smaller generation value means that it is added earlier than voxel with a larger generation value. In consequence, all pixels in the grown region with the same generation value form a surface as shown in Fig. 2. The surface described in Fig. 2 can be divided into a few isolated surfaces. As the word "isolated" indicates, voxels in different isolated surfaces are not connected to each other while voxels in the same isolated surface are connected to each other with the voxel having the same generation. The size, normal direction, and average gray-level of isolated surface are important properties which can describe the essential process of region growing.

2.1.2 Seed point and seed surface

Seed selection is the only human interactivity that region growing needs. There is no special constraint when selecting seed points besides the seed should be a typical voxel in the ROI (Region Of Interest). However, the region growing process will be greatly facilitated without increasing any human interactivity if we introduce a new constraint. Thus seed surface which is produced by a seed point is introduced. First we locate a relative uniform tube shape in ROI with clear boundaries to ensure the quality of seed surface. Then we select the tube's central point as the seed points which would not be a hard task when we deal with 3D cell axon datasets. Finally the original region growing is started as described in Fig. 3. After the growing surface touched the tube boundary, the growing surface will be automatically divided into two isolated seed surfaces with normal direction that parallels the tube's direction. Our self-adjust RG will start from these seed surfaces given by aforementioned approach.



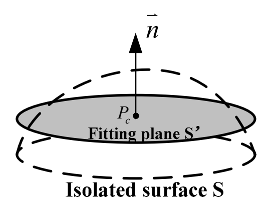


Fig. 3 Seed surface. Fig. 4 Fitting plane of isolated surface.

2.2 Surface modeling and information collection

Self-adjustment indicates our method is a two phase method. First, we track every evolution of region growing and collect selected information which can describe the evolution essentially. Then decisions are made to detect and stop errors. The information collection and update process is discussed in this section, and the realization of self-adjustment will be discussed in the next section.

After every evolution of region growing, the information collect phase is activated. First, isolated surface S of the current generation is detected and the corresponding best fitting plane S' [6] represented by $ax+by+z+c=0$ is calculated. Consider voxel $P_i=(x_i,y_i,z_i)\in S,\ i=1,\cdots,n$ with gray level g_i, to find the weighted best fitting plane in a least square sense, the function $\varphi=\sum g_i(z'-z_i)^2$ has to be minimized. Then we use surface S 's centroid $P_c=\frac{1}{n}\sum P_i=(x_c,y_c,z_c)$ which is contained by S' to solve for c and get:

$$\varphi=\sum g_i(ax_i+by_i+c+z_i)^2=\sum g_i[a(x_i-x_c)+b(y_i-y_c)+(z_i-z_c)]^2$$

The plane normal $\vec{n}=(a,b,1)$ can be found by calculating:

$$\begin{cases} \dfrac{\partial \varphi}{\partial a} = 2\sum g_i[a(x_i - x_c) + b(y_i - y_c) + (z_i - z_c)](x_i - x_c) = 0 \\ \dfrac{\partial \varphi}{\partial b} = 2\sum g_i[a(x_i - x_c) + b(y_i - y_c) + (z_i - z_c)](y_i - y_c) = 0 \end{cases}$$

Then the further analysis of the fitting plane is taken to provide information for decision making. Following properties of best fitting plane S' are collected and calculated:

(a) Shape information

As shown in Fig. 4, centroid P_c, surface's projected area A and normalized plane normal $\bar{n}$ of S' is calculated to form a propositional description of the surface.

(b) Gray-level information

Instead of recording every single voxel's gray level, we collect mean $\bar{G}$ of the voxels belong to the same isolated surface which delivers statistical property of surface.

(c) Corresponding isolated surfaces in previous generation

Because of the continuity of ROI (cell axon), we can use previous isolated surfaces to predict current isolated surfaces' properties and thus benefit the decision making. Considering the complexity and computational expense, we use generation m-1 and m-2 to predict generation m's normal direction $\bar{n}_m$:

$$\hat{\bar{n}}_m = \bar{n}_{m-1} + (\bar{n}_{m-1} - \bar{n}_{m-2})$$

After information which can essentially describe the region growing process is collected, the error detection phase is under taken.

2.3 The realization of self-adjustment

As stated previously, our region growing approach is a self-adjustment approach. In order to realize the self-adjust function, first we locate the errors need to be adjusted and then correct these errors.

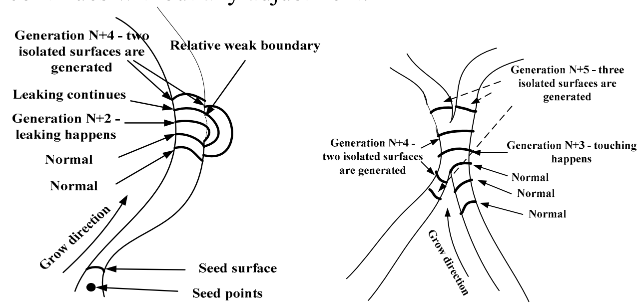
Generally speaking, there are two kinds of errors in cell axon region growing process. One is leaking and the other one is touching. The first error is caused by the region growing approach itself while the latter one is considered to be the problem of the target dataset. We will discuss these errors respectively.

(a) Leaking problem

Leaking happens at relative weak boundaries of ROI. Even with an optimal homogeneity criterion, the region growing can leak out if the contrast at the object boundary is not sufficient. As shown in Fig. 5(a), the region growing approach acts normally until generation N+2. The leaking happens at generation N+3 where there is a relative weak boundary denoted by the dotted line. There would be a very small branch of the extracted area at the initial stage of leaking (generation N+3). However, this branch will be expanded greatly (generation N+4) and produce very poor results after a few generation. So it is critical to detect the leaking as early as possible. The earlier we detect it, the easier it would be for us to remove the wrong branch caused by leaking. As the normal grown area and the leaked part will be departed by the strong boundary, multiple isolated surfaces can be detected after generation N+3.

(b) Touching problem

Touching problem is another prevailing phenomenon resulted by multiple closed axons which cannot be detached correctly in some region of the target dataset even by human. This kind of error is hard to detect when using traditional region growing methods which do not employ further analysis of the grown region and just take localized information into consideration. On the contrary, our self-adjust region growing analyzes the retrieved region after every generation and makes decision on a global basis. Fig. 5(b) points out when touching happens (generation N+3), another axon is added to grown region and thus multiple isolated surfaces are produced at generation N+4. Worse result (generation N+5) will be produced if region growing continues without any adjustment.



(a) (b)

Fig. 5 Leaking problem and touching problem.

After we review the properties of error patterns, we can find that error happens when multiple surfaces are detected (generation N+4). In that case, we need to select the correct isolated surfaces after every evolution of region growing if there are multiple surfaces. If correct surface is selected, our scheme can stop the error from spreading and adjust itself. It is possible to have multiple correct surfaces; however, in order to predigest the algorithm, we use only one surface to continue further evolution. Our surface selection algorithm can be described as follows:

For all isolated surfaces $S_1 \cdots S_n$ in generation m:

Step 1: remove S_x if $A_x < 0.1\sum_{i=1}^{n} A_i$, if there are more than one surface left.

Step 2: sort all surfaces according to project area A as $S_1, S_2 \cdots S_n$ from large to small, respectively.

Step 3: if S_1 is a primary surface which $A_1 > \sum_{i=2\cdots n} A_i$, select surface S_1 and go to step 7.

Step 4: remove all surfaces but three largest surfaces S_1, S_2 and S_3.

Step 5: remove S_x which gets most different $\overline{G}$ compared to previous generation.

Step 6: select S_x whose $\bar{n}_m$ is closest to the predicted value as candidate surface.

After the right surface is selected, the region growing continues and generation $m+1$ is produced.

3. RESULTS

The proposed approach has been validated by applying it to several representative volumes of axon images. It takes approximately 10 seconds per axon to automatically retrieve through 256 slices on a desktop PC with 1.8GHz CPU. The number of axons varies dynamically during the whole sequence.

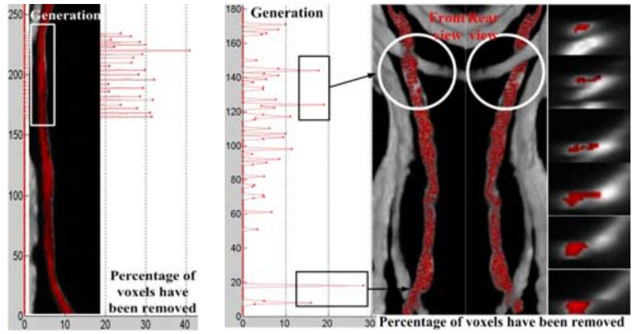
In Fig. 6, the partial result from a volume containing 128 slices is shown. An axon with a low contrast boundary is extracted in (a). In the marked area, the red curve which represents the percentage of voxels that have been removed shows about 25% of the grown voxels are removed from the grown region so that leaking can be stopped. Two touchings are detected and avoided in (b). Because of the tortuous nature of axons, our scheme removed some voxels by mistake; however, the red curve which gets two groups of peaks when touching happened demonstrates the validity of our scheme. The right most images show our scheme is able to choose the correct part of the touched axons although there is no palpable boundary in some slices.

Fig. 7 provides the testing result on a volume which contains 4 axons through 256 slices. The radii of axons are about 5-7 voxels, and the distance between axons is about 0-3 voxels. As shown in Fig. 7, we can successfully track all axons although there are several touchings. Some slices of the marked area are shown on the right.

4. CONCLUSION

This paper has introduced a novel neuron axon extraction approach with self-adjust ability for real-time optimization of 3D region growing results from image volume produced by serial block-face scanning electron microscopy. Our scheme is composed of three steps, a 3D region growing for getting a set of isolated surface, a modeling process for retrieving essential information from isolated surfaces, and the self-adjust approach for removing incorrect surfaces.

We have shown the validity of our scheme against a variety of interferences and the ability of extracting whole axon structures. Compared with classical approaches, our scheme shows much better results derived from global information explored and utilized. We also expect new improvements of our scheme and explore its application in other domains.



(a) (b)

Fig. 6 Processing results of leaking and touching.

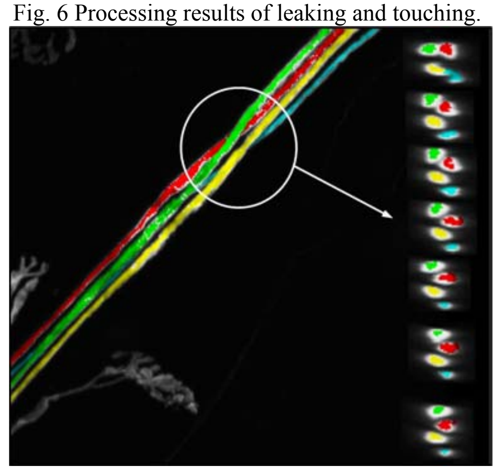


Fig. 7 Performance on long axons.

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