

Automated Analysis of Astrocytes in 3D Microscopy Data

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ABSTRACT

Astrocytes play a critical part in the regular functioning of the central nervous system and are widely studied to better understand how the body reacts to various diseases. Hundreds of individual cells must often be analyzed to produce statistically relevant analytical data from three-dimensional (3D) microscope images of Astrocytes. As a result, having an effective mechanism for automatically analyzing these data in a way that is resistant to human bias is critical and challenging because glial cells are generally uniform and densely packed. Therefore, we present an approach that automatically processes 3D micrographs of astrocytes and generates a set of measurements that can be used to compare between different images. We conducted an experimental evaluation of our approach based on a set of images of astrocyte 3D micrographs. The initial results show that the proposed approach is potentially promising.

Key words:

Astrocytes, Thresholding, Level Set.

1. INTRODUCTION

Astrocytes, commonly known as astroglia, are star-shaped glial cells that are mostly found in the brain and neurological system. Astrocytes play vital function in maintaining the blood-brain barrier (BBB) and they can be affected by diseases and traumas such as glaucoma [1], retinal detachment [2], and demyelinating disease [3]. Glial process retraction, as well as other structural alterations in astrocytes, can alter BBB permeability and have a major impact on disease development and infiltrate trafficking [4].

However, due to the cell's standard complicated form and intertwined processes from several astrocytes, examining this glia is a difficult endeavor. It is difficult to say with certainty which voxels belong to which cell. While existing visualization technologies

such as Light Microscopy, Electron Microscopy, and Confocal Microscopy offer high magnification capacity, they all need human analysis of hundreds of images, which is time consuming, vulnerable to operator bias, and difficult to do in 3D images.

In this study, we introduce a method to automatically analyze spatial astrocyte structure. The method is divided into three consecutives namely: preprocessing, segmentation, and analysis of segmented astrocyte. A set of preprocessing filters are used initially in our method to reduce noises if the input images. The cells' pictures are then divided into individual cells using a combination of two segmentation methods: thresholding and level set. Finally, the body of each cell is identified, and the branches of the cell are counted and measured.

2. PREPROCESSING

Preprocessing is usually necessary to improve the image in ways that increase the likelihood of future operations succeeding. The goal of this step is to improve the segmentation stage's results. It covers approaches for improving contrast, eliminating noise, and isolating regions in such a way that the noise is suppressed but the edge and detail information is preserved.

Two segmentation approaches are used in our method: thresholding and level set segmentation. The input image must be preprocessed before the segmentation process to get a better outcome in each approach. The only preprocessing step required for threshold segmentation is to transform the input image to an 8-bit type. Two preprocessing processes are required for level set segmentation: hybrid median filtering and erosion.

Hybrid median filtering is a noise reduction smoothing filter. The hybrid version of the median filter can have better performance in of maintaining

edges than the basic version because data from different spatial directions are ranked independently. The filter operates in three stages:

1. The reference pixel is used to determine the median of horizontal and vertical pixels.
2. The reference pixel is used to calculate the median of diagonal pixels.
3. The original pixel value is compared to these two values (median value from step 1, median value from step 2), and the median of these three values becomes the output value for the pixel in the filtered image.

Erosion is a basic mathematical morphology operator that is often applied to binary images. The primary idea is to get rid of any foreground pixels that are not surrounded by white pixels.

Before applying Level Set and trying to get the astrocyte characteristics in the segmentation step, the erosion effect increases the astrocyte and decreases the backdrop of the original image to overcome any leak in the image stack.

3. SEGMENTATION

In our technique, the user has the option of using thresholding or level set segmentation. Thresholding is perhaps the most straightforward and often used method for segmenting an image. It works in a nutshell as follows:

1. Determine a t threshold value.
2. If the value of each pixel in the input is less than t , set the value to 0, otherwise set to 1.
3. Begin the connectivity analysis pass by scanning the image from top to bottom, from left to right. The tool assigns a tag to the first object's pixel when it is discovered. The tag is a one-of-a-kind integer that will be carried by all pixels in the same item. Each time a new object's pixel is discovered, its 13 prior neighbors (9 on the upper slice and 4 on the same slice) are checked for an existing tag. The current pixel is then assigned the minimum tag found in the surrounding area.

The selection of an optimal threshold value is critical to the method's success.

The level set method works in the same way that a standard flood fill does. While extending the region, it constantly calculates the difference of the current

selection to the newly added pixels, and it stops if it exceeds a pre-selected gray value difference. Leakage is a concern with this method. The selection may leak to the outside of the object if the object's boundary contains a gap [5]. In this scenario, using thresholding to segment the astrocytes is a superior option.

4. ANALYZING THE SEGMENTED ASTROCYTE

Identifying the astrocyte's body, which is the center and largest component of the astrocyte, is the first step in examining it. The reason for this is that the thickness of the astrocyte's body is greater than the thickness of its branches.

To identify the astrocyte's body, we first need to set a threshold value t by doing the following steps:

1. For each non-zero voxel, we count how many consecutive non-zero voxels there are in six directions (+X, -X, +Y, -Y, +Z, -Z). For example, to count how many consecutive voxels whose values are not 0 in direction +X for voxel (10,10,10), we examine the following voxels: (11, 10, 10), (12, 10, 10), (13, 10, 10) and so on until we get to a voxel of value 0.
2. As a threshold value candidate, we choose the smallest value from the six directions.
3. We chose t as the highest value among all candidates.

Next, we examine the t consecutive voxels in the six directions (+X, -X, +Y, -Y, +Z, -Z) for each voxel whose value is not 0. We add the voxel to an array list as a body voxel if the values of all voxels in all directions are not 0.

Finally, we use the following procedure to identify all voxels within the body:

We create a cube shape window of size $(t2 + 1, t2+1, t2+1)$ around each voxel in the array list, with the voxel at its center. We examine each voxel within widow. If a voxel's value is not 0, then the voxel is inside the body:

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After eliminating the recognized body, the principal branches may now be easily enumerated. To examine the secondary branches, each primary branch is separated independently. The secondary branches are extracted using the skeleton approach [6]. Our method starts by skeletonizing each principal branch, then applying the skeleton method to the secondary branches branch that has been skeletonized based on their 26 neighbors, the voxels in this method are categorized into three categories:

- End-point voxel: a voxel with fewer than two neighbors.
- Slab voxel: A voxel with exactly two neighbors is called a slab voxel.
- Junction voxel: A voxel has more than two neighbors.

The slab segments, which connect end-points, end-points and junctions, or junctions and junctions, are the secondary branches based on these classifications. Figure 1 depicts a typical outcome of our technique for assessing a perfectly segmented astrocyte. To easy the visualization, the astrocyte's body and each primary branch is given a different color. In this example, all principal branches are identified and given various identification numbers ranging from 1 to 8. The number of secondary branches that correspond to each primary branch, as well as their length, are included in the study.

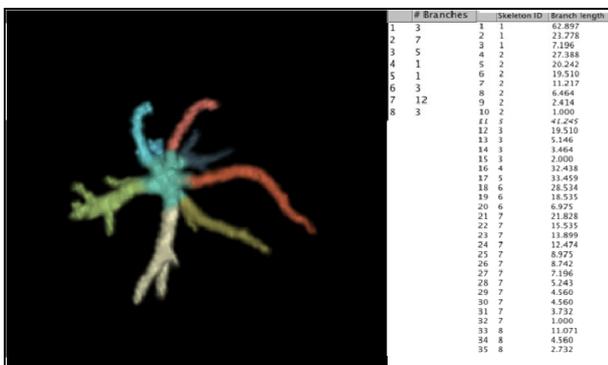


Figure 1. Astrocyte Analysis

The proposed technique was implemented in Java and packaged as a plugin for ImageJ [7] which is a Java-based image processing framework that is developed by the National Institutes of Health and commonly used in image processing research. Figure 2 shows a screenshot of the user interface.

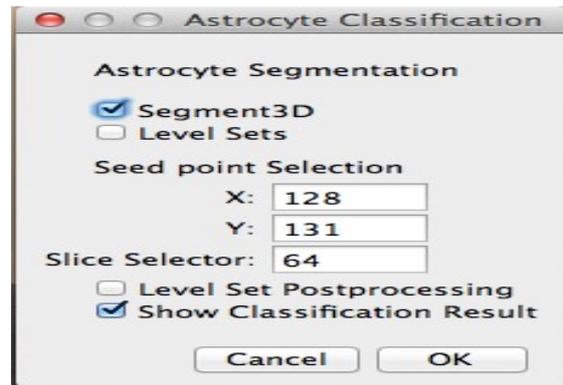


Figure 2. User Interface

5. EXPERIMENTAL EVALUATION

Frozen brains were fixed in 4 percent paraformaldehyde in PBS for 48 hours before being placed in PBS and stored at 4°C. They were then sliced at a thickness of 100 microns on a media cooled vibratome and stored at 4°C until utilized for the staining procedure. Tissue was immunofluorescently treated to stain astrocytes with a glial fibrillary acidic protein mouse anti-human GFAP-CY3 (1:500, Sigma, St. Louis, MO.) antibody in PBS for 24 hours. Triton- X is 5% of the total. Sections were dried on charged slides and cover slipped with Vectashield antifading mounting material (Vector Laboratories,

Burlingame, CA) after three 15-minute washes in PBS. With three laser lines, imaging was done with an Olympus laser scanning confocal microscope and related Fluoview software (Olympus America, Melville, NY). Using a 40X oil objective with an XY resolution of 256x256 and around 100 z sections, optical sections spanning the depth of the astrocytes were obtained.

Our method was tested on 19 photos of astrocyte 3D micrographs. Each photograph was tested twice. In the first test, we employed thresholding segmentation, and in the second test, we used level set segmentation. Using thresholding segmentation, 14 of the 19 pictures (73 percent) were accurately segmented. Figure 3 depicts some of the segmentation's outcomes. On the other hand, employing level set segmentation, only 9 photos (47%) were accurately segmented. Figure 4 depicts some level set segmentation findings. As a result of our research, we can infer that thresholding outperforms level set on our benchmark, successfully segmenting 25% more astrocytes. While leaking affects the accuracy of level set, thresholding is more sensitive to noise and choosing an ideal threshold can be difficult photos (47%) were accurately segmented. Figure 4 depicts some level set segmentation findings. As a

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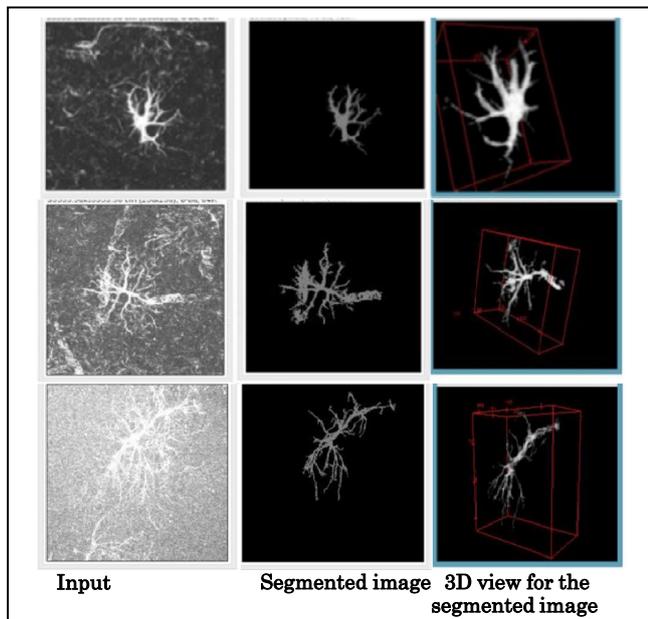


Figure 3: Thresholding Segmentation

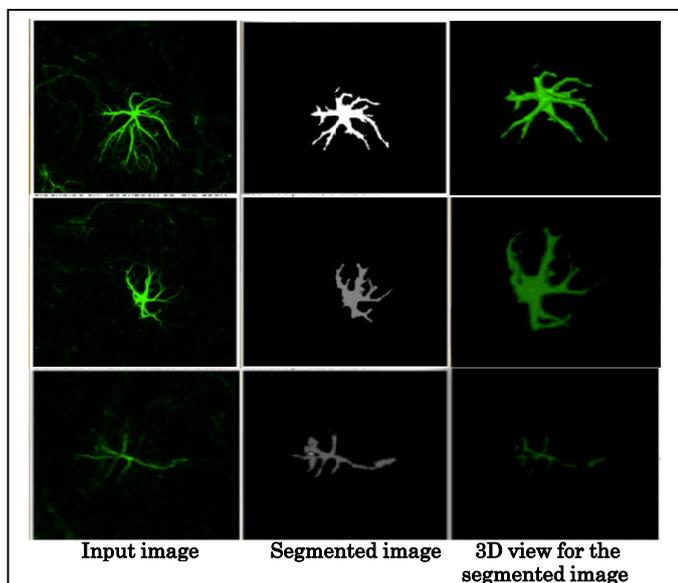


Figure 4: Level Set Segmentation

6. CONCLUSIONS AND FUTURE WORK

We devised a method for staining and automatically analyzing the 3D structure of astrocytes in this study. There are three steps to our strategy. The preparation of the astrocyte's input image to eliminate noise and simplify the image is the first stage. The image is then segmented to clearly distinguish the astrocytes in the second stage. The thresholding or level set approach can be used to segment the data. The astrocyte is next analyzed by identifying its body, counting the number of primary and secondary branches, and measuring the length of each branch in the third stage. The proposed method was tested on 19 photos and yielded promising results.

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References

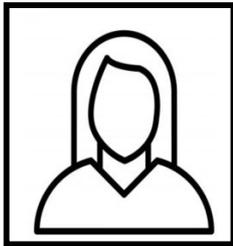
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