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建構互動式腦神經網路資料庫之神經基因表現圖譜及其三維影像處理系統——細緻結構之萃取與分析（子計畫三）

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ABSTRACT

We develop algorithms to extract the fine structures in the fly brain confocal microscopic images. In this year, we develop an algorithm and implement the code for 3D neuronal structure extraction. Comparing to the algorithm, the gradient vector flow snake method developed last year, the new algorithm needs much less user assistant. We also develop methods to segment the ellipsoid body. The accuracy of the segmented results is accurate enough to locate the center of that structure. Finally, we propose a possible coordinate system for the fly brain based on the principal component analysis and the segmented ellipsoid body.

1. Introduction

Our work emphasize on the fine structure extraction. The first task was to extract the structure of neuron from a set of confocal microscopic images. The segmentation of neuronal structure from confocal microscopic images or multi-photon microscopic images is an important task. We worked on this problem for many years. The first approach was to apply a vessel tracking algorithm. But the tracking often stopped due to sharp turn and low contrast of the neuron fibers. We then switched to algorithm that needs user assist. We then proposed an approach designed based on the GVF snake method. That method worked well even there are sharp turns along neuron fibers. The only problem with this approach was the needed of the user to provide an initial path. For some cases such as projection neurons, to provide the initial paths is a tolerable task. But there are cases that neurons are extremely complex so that to inputs the initial paths becomes impossible. In this year, we have developed a new method that can almost automatic extract the neuronal structure with the least possible use input- a point in the soma.

Since we are able to segment the ellipsoid body, we propose a Cartesian coordinate system based on the segmented ellipsoid body. We tried to align 9 fly brains into the coordinate system. It seems deviation between brains in the coordinate system is small. Based on this result, we also look for a coordinate system to describe the fine structures in the brain.
In the following section, we present our results obtained in this year.

2. Results

2.1. Neuronal structure segmentation.

The proposed method is designed based on an optimization technique in graph algorithms. The accumulated experiences of ours, we found it is difficult to trace neurons in 3D volume. It is also difficult to design a thinning algorithm in 3D space. But there are effective thinning algorithms in 2D space. From these observations, we developed a method that calculates the 2D skeleton of neuron in 2D space first. The true 3D skeleton of neuron is then computed from the set of 2D skeletons.

We briefly describe the algorithm in the following.

1. We compute the skeleton of neuron in each slice. Note that although the neuron is a connected component in the volume data, there could be more than one connected component in a slice.

2. For the skeleton in each slice, we determine the set of end points in the skeleton. Note that, an end point may or may not be a true end point of the neuron fibers in 3D space. If a 2D end point is not a 3D end point, this point is generally close to the neuron fiber that moves from one slice to the other. We set a deviation that can eliminate such kind of points.

3. The third step is to compute the neuronal structure from the set of 2D skeleton. We assume that the center point, \( p \), of the soma is available. Suppose that we have the shortest path from between all of the points to \( p \). The farthest 2D end point, \( q \), from \( p \) should be the true 3D end point. We compute the path from \( p \) to \( q \) and trim the 2D end points that are close to the path from \( p \) to \( q \) (since there are likely not to be the true 3D end points). This process is carried out iteratively. In each iteration, we compute the longest path from \( p \) to a 2D end point. We then trim out those 2D end points close to the path. This process iterates until the set of 2D points is empty. The method for constructing the graph is stated in the Appendix A.

This method has been applied to more than one hundred sets of data. In almost all of the cases, we could achieve very good result. Only a small portion of the data sets could not be done successfully. We believe those data sets were acquired by an individual and the quality of that images are much worse than the others. The figure 1, 2 and 3 show the tracing results.

![Figure. 1. The projection neuron in *Drosophila*’s calyx](image-url)
Figure 2. This figure shows the *Drosophila*'s projection neuron in the lateral horn.

Figure 3.

### 2.2 Segmentation of the ellipsoid body.
Segmentation of neuropils is important in fly brain research. Professor Chen with Tsing Hua University (our project leader) has developed method to segment the mushroom body in the fly brain. In this project, we tried to segment the ellipsoid body and the antenna lobes. To segment the neuropils, the user has to provide a bounding box that encloses the region of interest.

The segmentation algorithm is developed based on the intensity difference between the region of interest and the background. To segment the ellipsoid body, we use the property that the shape of
this neuropil is a donut shaped object. To segment the antenna lobes, we use the property that it is a spherical shaped object. We then determine the voxels in the region of interest.

Once the voxels in the ellipsoid body is determined, since it is donut-shaped, we can compute a point which is the best approximation of the center of the ellipsoid body. The center of the ellipsoid body is defined to be the center of two co-centric circles, C1 and C2. Both of C1 and C2 are cantered at the center of the ellipsoid body and C2 enclose C1. C1 is the largest empty sphere and C2 is the largest enclose sphere of the ellipsoid body. Two segmentation results are presented below.

![Figure 4](image)

**Figure 4**

![Figure 5](image)

**Figure 5**

Figure 4, 5. The structure colored by yellow is ellipsoid body. Note the red structures with high intensity which is close to the ellipsoid body are parts of mushroom body.
2.3. A possible coordinate system
Since the ellipsoid body is between the two half brain and the center of the ellipsoid body can be defined, we attempted to design a Cartesian coordinate system centered at the center of ellipsoid body. The three axes are obtained the principal component analysis of the gray scale volume data. Given two fly brains, we compute the center and the axes for each brain. This two brains are aligned so that

1. Their centers are coincide and
2. Three axes are aligned.

Since axes and center are defined, a bounding box enclosing the brain can be defined. The bounding box serves as a scaling parameter to scale up or down of the brain. We have aligned 9 brains into a box. Our results, figure 6 and 7, show that, even without deformable warping, the internal structures of the fly brain are also aligned.

Figure 6
3. Conclusion and Discussion
We developed neuron tracing method that needs very little user assistance. The user only has to define a point in the soma which is root in the neuronal tree. The method shall be applied to trace neuron in eight hundred sets of data. We also develop method to segment neuropils in the fly brain. We allow a few user interface operations so that biologist can segment the neuropil efficiently. Based on the segmented neuropils, we shall propose a coordinate system in which we can define the location of neurons and other structures.

Appendix A

For every slice we choose a threshold based on its intensity histogram and then binarized the image slice. A refined binary image stack, \( V_b \) is then obtained. A 3-D 26-neighbor connected component analysis is applied. In most of the cases, the largest connected component is the desired neuron. Let \( V \) be the volume containing the binarized neuron. The Euclidean distance transform is then applied to each image slice in \( V \) and construct the skeletons, \( S_k \) of every object in the foreground of slice \( k \). For each slice \( k \), we compute a set of candidate 3-D end points by examining 9 digital planes in the 26-neighborhood of each end point of \( S_k \). The set of skeleton points in each slice plays an important role in designing the potential function. The set of candidate end points is denoted \( E^0 \).

In order to make the path lies in the center of the desirable structure, we define the potential as an awarding function \( f \) as follows. \( V \) can be considered as a grid graph that the vertices are voxels and the edges are defined by the connected components in \( V \) and its neighbor \( q \), there is an edge defined by the pair of connected vertices \( (p, q) \), \( f(p, q) \) satisfies the conditions:

1) \( f(p, q) < 0 \) if \( q \in S_k \), for some \( k \), otherwise, it equals 0
2) Let \( \text{Dis}_{\text{Euclidean}}(p, q) \) be the Euclidean distance between \( p \) and \( q \), \( \lambda |f(p, q)| < \text{Dis}_{\text{Euclidean}}(p, q), \forall q \in S_k \), for some \( k \). Under the second restriction, we can guarantee that there are no negative edges in the weighted grid graph of \( V \).

By applying the awarding function to deduce the minimal path from a given source point, \( s \) is as follows. From the given source point, \( s \), we apply the well-known Dijkstra’s algorithm to calculate the single source shortest paths to all the other end points.

Papers published or submitted