Template-based automatic segmentation of *Drosophila* mushroom bodies

Guan-Yu Chen, Ying-Cheng Chen, Chu-Fang Lin, An-Chi Hu, Cheng-Chi Wu, Yung-Chang Chen *
Department of Electrical Engineering
National Tsing Hua University, Hsinchu, Taiwan, R.O.C.
{cgu,dennis,libra,ycchen}@benz.ee.nthu.edu.tw

ABSTRACT

The segmentation of *Drosophila* mushroom bodies is highly demanded in the *Drosophila* brain research. In this work, we developed a template-based segmentation system with integration of the 3D average brain model. 3D registration and warping are performed to derive initial contours for the follow-up segmentation procedure which uses the snake algorithm. 3D models can be reconstructed from the segmentation results to update the average brain model. The proposed system can perform the segmentation fast and automatically to facilitate the construction of a more precise standard 3D *Drosophila* brain model.

1. INTRODUCTION

Constructing a representative 3D *Drosophila* brain is significant to the research of *Drosophila* learning and memory. A precise standard *Drosophila* brain model acts as a reference template to integrate experimental data from different laboratories. Consequently, it is essential to build more 3D brain models from different flies to ensure the representativeness of the 3D *Drosophila* brain model. However, this task is used to be slowed down by the great amount of manual interference required to segment neuropils such as mushroom bodies, antennal lobes, and central body complex in confocal microscopic images full of noises. The difficulty of rapid generating *Drosophila* brain models resulted in inadequate *Drosophila* brains available for average brain model in the previous work [1]. Therefore, the automatic and rapid construction of individual 3D *Drosophila* brain models from each set of confocal microscopic images is highly demanded. The system we developed allowing automatic and rapid segmentation of the mushroom bodies meets the need and considerably boosts the speed of generating 3D *Drosophila* brain models. This will consequently provide more available brain models to construct a representative standard brain model.

This paper is organized as follows. Section 2 overviews the proposed system. Section 3 describes the 3D registration, segmentation algorithms used in our system, and the techniques designed to reduce the disturbance of noises. In Section 4, the result of both 3D registration and segmentation are presented, followed by the conclusion.

2. OVERVIEW OF THE SEGMENTATION SYSTEM

The flowchart of our system is illustrated in Figure 1. It encompasses two major procedure, automatic contour initialization and segmentation using snake algorithm.

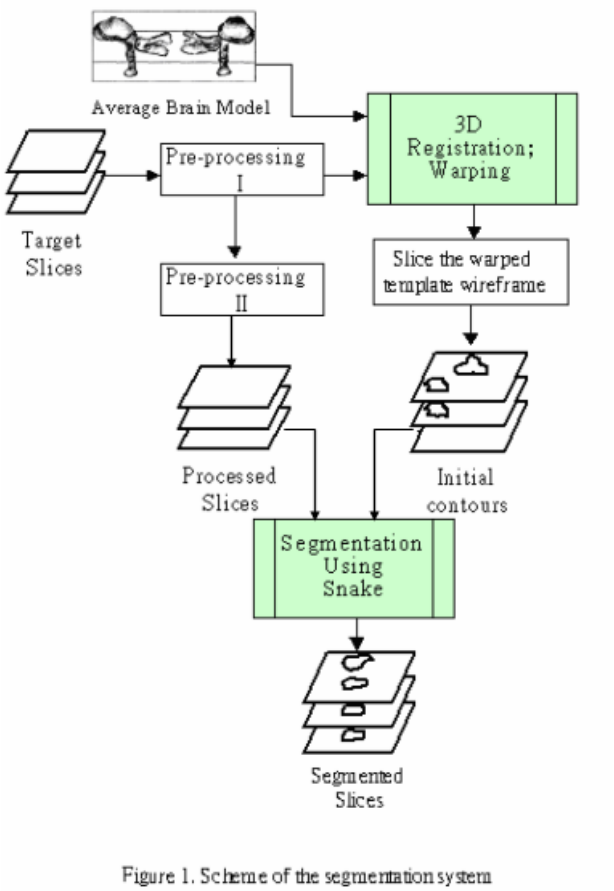


Figure 1. Scheme of the segmentation system

The average brain model acts as a template wireframe. For each individual set of confocal microscopic image slices (Target Slices in Figure 1), we first construct its 3D wireframe model and then register the average brain model to the target one. By further warping the template

wireframe and then slicing it at the corresponding position of target slices, we derive the initial contours of mushroom bodies in each target slice. In the procedure of segmentation, snake algorithm is used to evaluate the correct boundaries of mushroom bodies in each slice. Due to the severe disturbing noises coming from the dyeing of other surrounding neuropils and cells in the experimental images, specific techniques are used to reduce noise in both the 3D registration and segmentation procedures.

3. ALGORITHMS

3.1. Automatic Contours Initialization

In this section, we first register the average brain model to the target 3D wireframe model. Subsequently, we slice the transformed average model to the corresponding position of target slices to derive the initial contours of each slice automatically.

The average model was generated by two-level model averaging techniques described in [1]. This method partitions each individual model into several significant parts manually with a user interface, and computes principal axes of these parts by the technique of PCA (principal component analysis)[2]. A set of principal axes is extracted and then performed the averaging technique (Figure 2). The generated average model which consists of information about six axes of mushroom bodies can be used to implement the warping step.

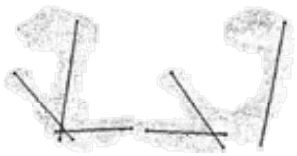


Figure2. The principal axes of the average model

The automatic initialization algorithm can be divided into three major parts:

3.1.1. Pre-processing I. Due to the process of imaging, the initial state of mushroom bodies may be different. Pre-processing step, which includes denoising, initial rotation and scaling, not only let the target mushroom bodies and standard model have approximately the same direction and scale, but also delete some noises. In this way, the result of global registration will be improved.

We use classification method to delete noises (Figure 5) which do not contact with mushroom bodies. A rectangular parallelepiped which contains the mushroom bodies is defined to be the bounding box. The parallelepiped is constructed using PCA technique to decide its axes. We assume that the direction of the length of the bounding box is equal to the eigenvector of the largest eigenvalue, which is computed from the covariance matrix of the target 3D wireframe or standard model. So we can calculate the angle between the two bounding box and use rotation matrix to modify the angle difference of horizontal lobe between target 3D wireframe and standard model. And we also transform the bounding boxes to the same scale. The result is presented in Figure 6.

3.1.2. Registration. The registration includes the global registration and the local registration.

Global registration:

In this step, we want to register the whole part of standard model to the target 3D model. The affine registration is chosen for the purpose, and the differences such as global position, rotation angle, scale, and shearing will be reduced.

After the global registration, we divide the transformed standard model into two parts and perform the local registration independently.

Local registration:

1. Apply affine registration again: Since the global registration is a rigid transformation, its performance is not guaranteed if there is an angle difference between left and right parts of the target 3D model. Applying the affine registration to the two parts independently can improve registration results (Figure 7).
2. 3D Field-based Warping: A 3-D field-based warping algorithm [3] is applied to warp the three axes of half standard model to those of half target 3D model. The warping function is defined as:

$$W(\mathrm{p}) = \mathrm{p} + \frac{\sum_{k=1}^{r} w_k \Delta \mathrm{p}_k}{\sum_{k=1}^{r} w_k}, \Delta \mathrm{p}_k = W_k(\mathrm{p}) - \mathrm{p}$$

where **p** is the position vector of vertices on standard model, and r is the number of principal axes(r =3). $W_k(\mathrm{p})$ is the warped position of **p,** which is defined as:

$$W_k(\mathrm{p}) = (\frac{\mathrm{distance(p)}}{\mathrm{t_{min}}}) * (\delta * \nabla(\mathrm{M}))$$

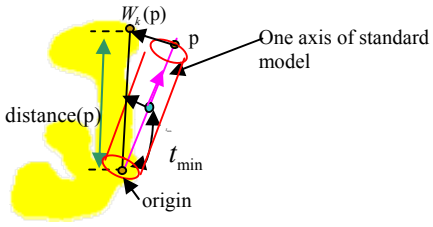


Figure3. Illustration of $W_k(\mathrm{p})$

where distance(p) is the distance from p to the assumed origin(the bottom of each axis). $t_{\min}$ is the distance from

the middle point of an axis to the origin. M is the middle point of an axis and $\nabla(\mathrm{M})$ is the average gradient of the vertices which are around M. δ is the iteration step size of warped position (Figure 3). By this way, M will move inside the target until it reaches the center of the target where $\nabla(\mathrm{M})$ is minimum. Then, other vertices of the standard model will warp according to $W_k(\mathrm{p})$.

The result of warping is shown in Figure 8.

3.1.3. Generation of initial contours. The warped standard model is sliced at corresponding positions of target slices and the contours are set to be the initial contours for the snake algorithm.

3.2. Segmentation Using Snake

3.2.1. Pre-Processing II. In pre-processing I, only noises at a distance from the mushroom bodies can be removed. Noises containing impulse points nearby the mushroom bodies and the adjacent cells are still remained. In order to keep those ones from affecting the calculation of the correct contour positions, it is indispensable to reduce them. This is done by grayscale morphological methods and the statistic information evaluation of the intensity around the mushroom bodies. At the position around the Calyces, mushroom bodies are particularly hard to be detected owing to being covered by lots of noises. It is observed that many cells nearby the Calyces are dyed and compose impulse noise. Using our method which first calculates the distribution of intensity and makes statistical evaluation, a particular threshold corresponding to each pixel on each image is derived. Performing thresholding according to the position-depended threshold reduces a great amount of noise around the Calyces and maintains the completeness of the mushroom bodies.

Another problem is the extended nerve fibers of central complex dyed surrounding the regions of mushroom bodies, which present similar intensity with mushroom bodies. In the slices containing both sections of central complex and mushroom bodies, grayscale morphological method is adopted and integrated with statistical thresholding to remove the noises. Using the morphological and statistical combined method, we can also reduce the noise from various neuropil regions surrounding the mushroom bodies (Figure 9). This pre-processing procedure effectively reduces the random noises that affect the evaluation of contour positions.

3.2.2. Snake algorithm. Once the initial contours for each slice are generated and the noises are reduced, we perform the snake algorithm to calculate the best position for the segmentation contours on each corresponding slice. The correct position of the final contours should locate at the outlines of the mushroom bodies. This is achieved by calculating the energy near the initial contour points, which is the weighting sum of continuity energy $E_{cont}(v_i)$, curve energy $E_{curve}(v_i)$, image energy $E_{image}(v_i)$, and constraint energy $E_{constraint}(v_i)$. The energy equation is described in (1). Each w represents the weighting of the corresponding energy term and is determined by the property of the images. Due to different sets of confocal microscopy slices may have image distinct qualities, the weightings is not constant.

$$E(v_i) = w_1 E_{cont}(v_i) + w_2 E_{curve}(v_i) + w_3 E_{image}(v_i) + w_4 E_{constraint}(v_i) \quad (1)$$

By moving the contour points to those with the minimal energy, the contours are attracted to the outlines of mushroom bodies (Figure 4). After the final contours derived, we can take these contours as a mask to segment the regions inside them and obtain a clean set of slices showing only the mushroom bodies. The segmented result of one of the slice image is shown in Figure 10.

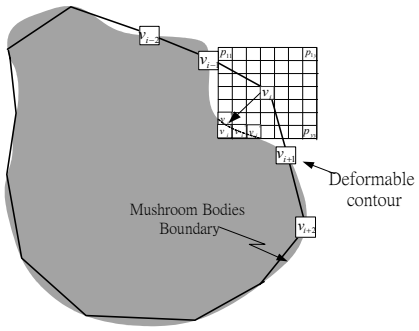


Figure 4. The contour is attracted to the boundary

4. EXPERIMENTAL RESULTS

Each individual set of confocal microscopic image slices contains about 140 slices, which include different sections of *Drosophila* brain image after dyeing. Figure 5 through Figure 8 illustrate the results of each stages of registration. After warping, the angles of the six axes of the standard model are approximately the same with those of target 3D wireframe. Figure 9 illustrates the result of using the morphological and statistical combined method to reduce the noise coming from various neuropil regions surrounding the mushroom bodies. Figure 10 illustrates the result of the final contours on the original image and the slice containing only the segmented mushroom bodies.

Figure 5. The result of "denoising", the left side is original target 3D view

(a) before pre-processing (b) after pre-processing

Figure 6. The result of pre-processing (original standard model is in red)

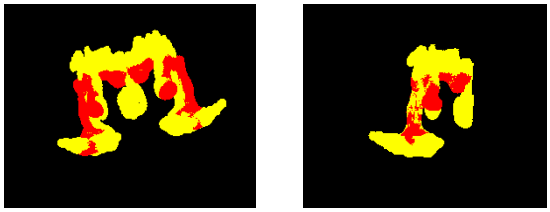
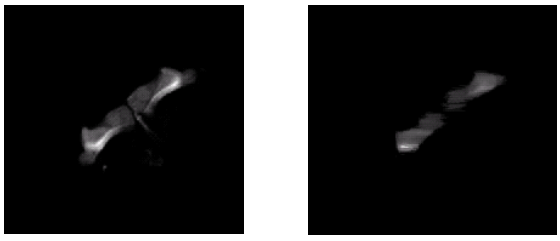


Figure 7. (a) after global affine registration (b) one side of (a) and after affine registration again

(a) before warping (b) after warping

Figure 8. The result of warping

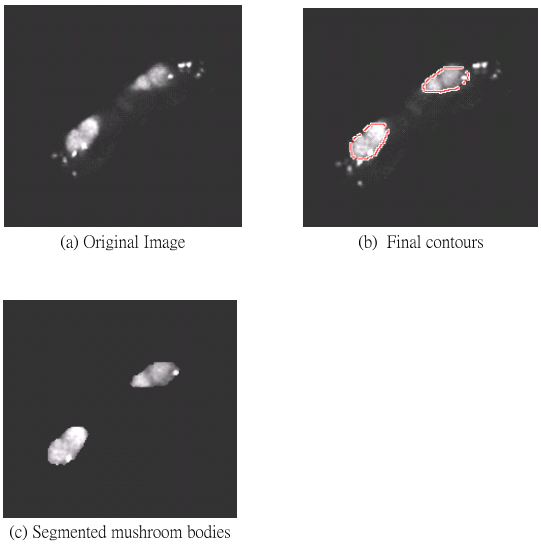


(a) Original Image (b) Processed Image

Figure9. The result of pre-processing II

5. CONCLUSION

In this paper, we develop a specific system for *Drosophila* brain segmentation and 3D *Drosophila* brain model construction. The system integrates the previous model averaging algorithms and provides a less laborious method to improve the average brain model, which is instrumental to speed up the generation of a standard 3D *Drosophila* brain model.



(a) Original Image (b) Final contours

(c) Segmented mushroom bodies

Figure 10. The result of segmentation using snake

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