FLYBOW IMAGE SEGMENTATION
For Tracing Neuron Circuits in Drosophila Brain

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Abstract: Recently developed were the Brainbow and Flybow techniques that can image and visualize a large number of neurons at a time. These techniques provide a way for imaging multiple neurons at the same time, and ideally, neurons can then be differentiated from each other according to their color information. However, due to dozens of neuron fibers spreading spatially in a very intricate structure, it is time-consuming to label them by hand and also difficult to trace them by using existing algorithms designed for tracing a single neuron. We proposed a prototype scheme based on grayscale morphological operations for segmenting Flybow imagery. The proposed method can provide segmentation results semi-automatically, and thus it would be useful for biologists to identify the neuro-circuits and develop the ground truth as well.

1 INTRODUCTION

One of the formidable challenges in neuroscience research is to understand how the information travel, encode, decode, and compute in the brain. *Drosophila* is a widely used genetic model system for understanding human biology because of its rapid generation time and the ease with which it can be handled in the laboratory (Bier, 2005). Simple brain circuits for intricate behaviors, most sophisticated genetic tool box and complete genomics and proteomics information make *Drosophila* an idea model system for studying basic mechanisms underlying the brain's operation. A key step towards understanding the development and function of the central nervous system is by characterizing the connections among neurons, which are exceedingly complex and yet precise in the central nervous system.

Recently developed were the Brainbow (Livet et al., 2007) and Flybow (Hadjieconomou et al., 2011) techniques that can image and visualize a large number of neurons at a time. Based on the combinatorial and stochastic expression of multiple fluorescent protein variants—for example, AcGFP for green fluorescent protein, CFP for cyan, mKO for orange, and YFP for yellow—from a single transgene, each neuron can be randomly assigned to a color via multi-copies reporters while being imaged. This kind of techniques not only lights the way to discriminate different neurons in a defined group of cells, but also provides an opportunity of tracing neural circuits in a single cell level. However, it is difficult to separate and trace the neurons due to the local denseness of neuron fibers and the signal crosstalk at imaging stage, and hence reconstructing the neuro-circuits becomes a burdensome task.

In this paper, we propose a prototype procedure for segmenting the neurons from the Flybow image stack of the Drosophila brain. The rest parts of this paper are organized as follows. In Section 2, the background and the related work about Flybow are briefly depicted; then in Section 3, the proposed method is described. We demonstrate the experimental results in Section 4, and finally we draw our conclusion and discuss the possible future improvements in Section 5.

2 BACKGROUND

Flybow technique provides a way for imaging multiple neurons at the same time, and ideally, neurons can then be differentiated from each other according to their color information. As shown in Fig. 1, each neuron—including its cell body and its fibers—is represented by a certain color, and therefore the neuron connections are hopefully traceable. There are many works studying how to trace neuron-fibers

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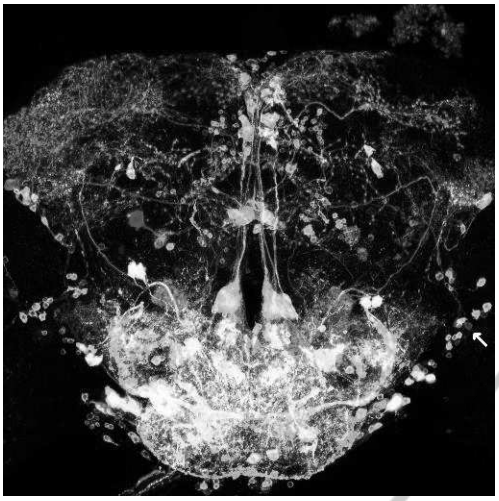


Figure 1: Top view of the image stack acquired by using Flybow technique. This image is obtained by projecting all slices to the XY-plane, and one of the cell bodies is pointed out with the arrow.

(Lee et al., 2009; Rodriguez et al., 2009; Peng et al., 2011), but few literatures address how to segment and trace the neuro-circuits acquired by Flybow/Brainbow techniques. So far as we know, only Bas et al. (2010) proposed a cylinder-shape-based method to trace a bundle of neuron fibers from Brainbow imagery. However, as to the case of dozens of neuron fibers spreading spatially in a very intricate structure, it not only seems impossible to segment neural circuits via cylinder-shape-based method, but also difficult and time-consuming to trace them manually. As the example shown in Fig. 2, it would be very laborious to identify each independant neuron in regions where the neuron fibers are dense because the voxel chrominance/luminance may be contributed from all nearby neurons. Furthermore, since the wavelengthes of green light, produced by GFP, and yellow light, produced by YFP, are so close, the resulting crosstalk in G-channel would also lead to color shifts. Therefore, it is not as intuitive as we anticipated to segment each individual neuron cell from Flybow image stacks. Our goal here is to develop a plain and easy-implemented prototype scheme for Flybow imagrey segmentation, and we expect to develop a robust unsupervised method after further studies.

3 SEGMENTING NEURONS

The key concept of the proposed method is to identify the locations of neuron cell bodies first and to trace the neural pathway from each of them afterward. There

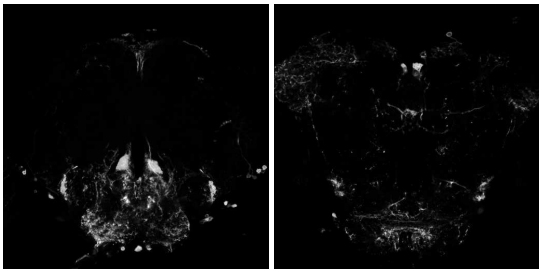


Figure 2: Two axial slices showing that some regions are dense with neuron fibers.

are two reasons for adopting this strategy. First, it is tough to recognize each individual neuron by considering color information since fluorescence of different wavelength may crosstalk and also attenuate over time and depth. Second, a neuron fiber is usually nothing more than a thin line/curve or a small spot on 2D image slices, whereas a cell body is often a round-/oval-shaped disk or a torus. Accordingly, it would be more feasible and systematic to find the cell bodies at the beginning and then to segment the neuron fibers thereof.

The proposed segmentation procedure is performed in somewhat divide-and-conquer style. Specifically, each of the R-, G- and B- channel is processed separately, and for a given channel, the location of every cell body is identified first, and the fiber of each neuron is traced independently in the next place. An additional consideration of adopting this strategy is that the segmentation result of a channel, e.g. R-channel, can be used to validate that of another, e.g. G-channel. It is because of that fibers in the channel, which suffers from crosstalk, is hard to be traced, and the proposed scheme can at least provide a circuitous solution to this kind of problem.

In the following subsections, we will first describe the algorithm overview and then state how to preprocess the source images. Succeedingly, introduced are the ways to extract cell bodies and to trace neuron fibers.

3.1 Algorithm Overview

Step-0: Separate the source images into R-, G- and B-channel images, and perform preprocessing.
Step-1: *Erode* each of the three channel, and then *reconstruct* the obtained masks images.
Step-2: Subtract the reconstructed images from the original ones.
Step-3: *Label* the obtained segmentaion masks, and then remove the irrational ones.
Step-4: Based on the original image and the results of Step-3 and Step-0, perform *grayscale morphological*

reconstruction again, and then trace the neuron fibers from each cell body via the just reconstructed images.

3.2 Preprocessing

The source images have to be preprocessed so that the edges of neuron fibers can be enhanced and the halation effect can be reduced. It is straightforward to enhance the pathway of neuron fibers by using high-boost filtering, but the high-boost operation may magnify the halation effect. The halation effect results from the fact that the fluorescence emitted by the neuron cells may halo the neighboring areas, as what can be observed in Fig. 1 and Fig. 2. Accordingly, we apply white top-hat transform, defined as the difference bwteen the input image and its morphological opening result, to reduce the halation. Notice that since the white top-hat transform can extract voxels brighter than their surroundings, the obtained result of this step is conceptually a skelonized image stack that would be a suitable input for trancing stage.

Remind that both high-boost filtering, also known as unsharp masking, and top-hat transform are conventional operations in image processing. Further, the details of these two operations can be found in (Gonzalez and Woods, 2007).

3.3 Extracting Cell Body

This step is primarily accomplished by morphological operations because cell body regions are likely to survive after several times of erosion, but neuron fibers are not. This step consists of three components, they are (1) erosion, (2) labelling, and (3) reconstruction. All procedures in these components are operated three-dimensionally, and the aim here is to find the 3D segmentation masks for cell bodies.

3.3.1 Erosion

Instead of general binary erosion, we adopt grayscale erosion which can gradually darken the input images so that it is advantageous not only to remove the neuron fibers, but also to locate the cell bodies. As illustrated in Fig.1, the fluorescence intensity of cell body region is usually over-saturated; therefore the intensity difference between original input images and the morphological reconstructed images can be used to indicate the positions of cell bodies.

3.3.2 Labelling

In this substep, the connected-component labelling is performed. The goals of labelling here are twofold: (1) remove the segmentation masks that are too small

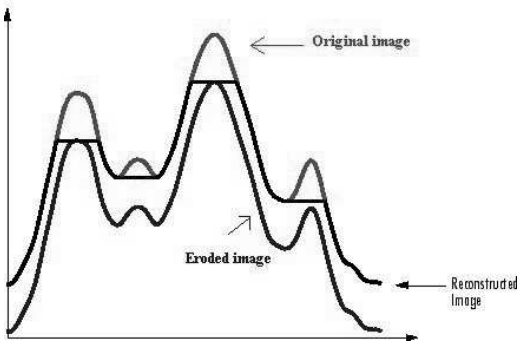


Figure 3: Illustration of the intensity decrement after grayscale erosion and reconstruction. The original figure is downloaded from Mathwork's website (Mathwork).

to denote cell bodies, and (2) discard the segmentation masks that contain cavities inside, as the arrowed area in Fig.2. The first case may happen when the segmentation is associated with dendrite or synapse, and the second case may related to the region where the neuron fibers are dense.

3.3.3 Reconstruction

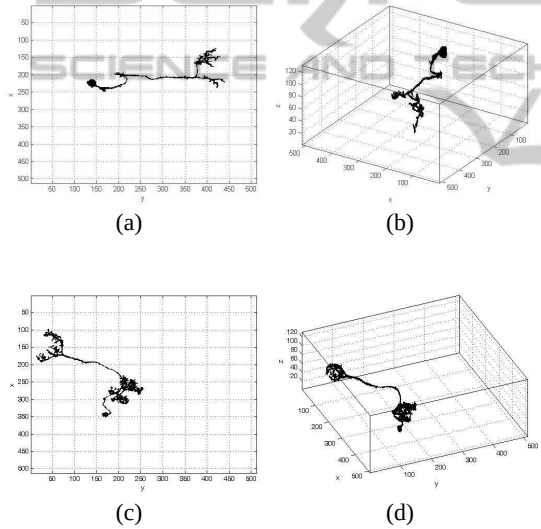
Based on the previous steps, the segmentation mask can be reconstructed by using grayscale morphology reconstruction. Then, the cell bodies can be located according to the intensity decrements obtained by subtracting the reconstructed images from the original ones. Additionally, the reconstruction result can also be used as a reference to evaluate the threshold for tracing neuron fibers, since it provides a feasible minimal intensity value for the given object, as illustrated in Fig.3.

3.4 Tracing Neuron Fibers

Based on the location of cell body, we then can trace its fiber by considering the spatial connections. Since each of the R-, G-, and B-channel is processed independently, the color information can be disregarded and only the spatial connectivity is considered. The proposed strategy is to start tracing from a cell body and then to connect recursively all the 26-neighbors in three-dimensional space until there is no more voxel can be connected. In other words, it is exactly the concept of region-growing. The threshold required for deciding whether a voxel should be connected is a user-specified parameter, although it can also be estimated from the morphological reconstruction result as we just described in the Subsection 3.3.3.

4 EXPERIMENT RESULT

The source image stack we used consists of 131 image slices of dimension 1024×1024, and the sampling resolutions along x-, y- and z-direction are respectively 0.35, 0.35 and 1.0 μm. Based on the amount of cell bodies that were extracted in our experiments, there are about more than 100 neurons successfully imaged and visualized in this image stack. Also, according to the biologists, almost all of neurons in this area—theoretically about 70000 neurons—are likely to be interconnected. Consequently, our goal is to extract and isolate independent neurons and their fibers from the flybow imagery as possible as we can. The proposed method is applied on the downsampled image stack with dimension $512 \times 512 \times 131$, and parts of our segmentation results are demonstrated in the following figures.

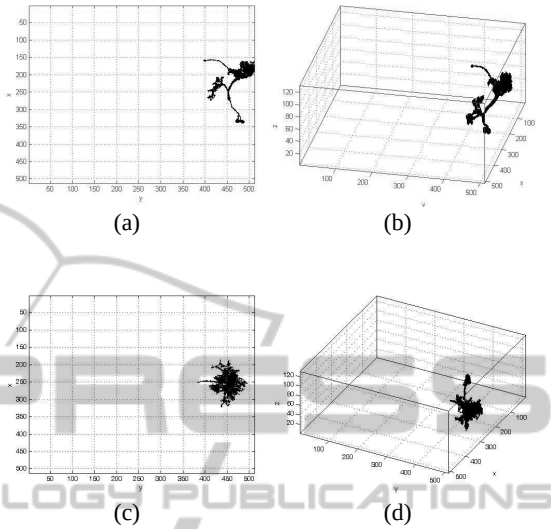


(a) (b)

(c) (d)

Figure 4: Different views of two independent neuron cells and the fibers thereof.

In Fig.4, two neurons are segmented successfully, and the segmentation result can then be used to picture how neuron fibers route in Drosophila brain in a single cell level. Take the neuron shown in Fig.4(a) for example. Its cell body locates approximately on $(225,130,117)$; its neuron fiber is initially extended toward the position $(199,208,95)$ and then turns to extend horizontally toward the place $(206,374,95)$; finally, one of its branches moves toward $(161,423,51)$, whereas the other keeps lengthening horizontally. In Fig.5, two neurons are segmented and traced well via the proposed method, even though fibers of two independent neurons are spatially entangled with each other. Comparing Fig.1 and 6 respectively with Fig.5(a) and (b) and Fig.5(c) and (d), it is easy to find that the neurons shown in Fig.5 are visualized in dissimilar colors, and hence they could be seperated according to their color information.



(a) (b)

(c) (d)

Figure 5: Different views of other two neurons. Note that the cell body of the neuron shown in (a) and (b) is exactly the one arrowed in Fig.1, whereas (c) and (d) is the one highlighted by label-2 in Fig.6.

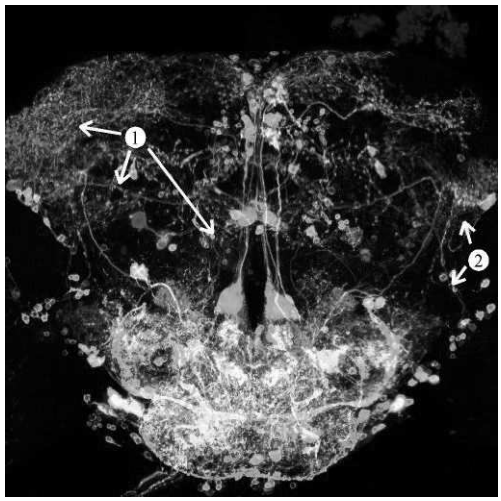


Figure 6: Label-1 indicates the neuron shown in Fig.4(c) and (d), and label-2 points towards the neuron shown in Fig.5(c) and (d).

Finally, Fig.7(a), (b) show two or three neurons that cannot be differentiated due to crosstalk or improper tracing threshold; meanwhile, Fig.7(c), (d) illustrated one another neighboring neuron which is visualized in different color and hence segmented suc-

cessfully. Fig.7 represents that even if the neurons, which are spatially close and randomly assigned to similar colors, cannot be clearly separated, the proposed algorithm can at least provide a reference to assist biologists identifying the neural circuits in a cell-to-cell level. In short, the experimental results show that the proposed scheme can segment the Flybow imagery well, even though there are still some improvements needed to be carried out.

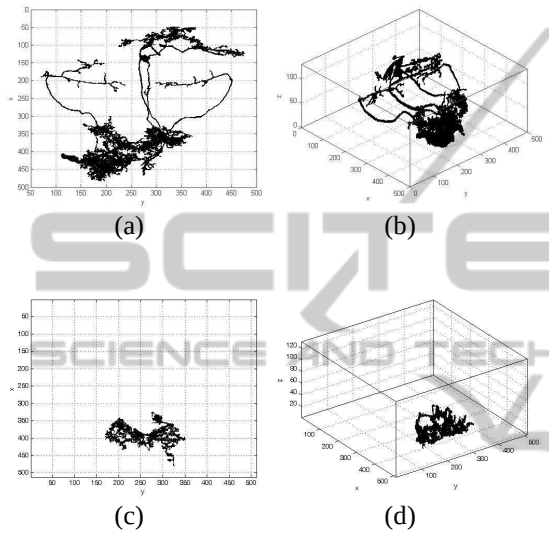


Figure 7: Neurons that cannot be separated from each other. Though terminals of the neuron fibers of at least 2 neuron cells are interlaced, the segmentation result can also provide a reference to biologists for identifying different neurons.

5 CONCLUSIONS

We proposed a prototype scheme based on grayscale morphological operations for segmenting Flybow/Brainbow imagery. It is time-consuming to label the neural circuits from Flybow/Brainbow imagery by hand and also difficult to trace them by using existing algorithms designed for tracing a single neuron. The proposed method can provide segmentation results semi-automatically, and consequently it would be useful for biologists to identify the neuro-circuits.

Besides, in order to develop a sound and robust algorithm for this kind of data, it is inevitable to establish a ground truth first. Thus, our segmentation results need to be verified by biologists repeatedly untill a well-accepted ground truth is constructed. We will start this task by first segmenting some neurons well-known in biological literatures and then extend the algorithm to other neurons. Moreover, there is at least one another reachable future improvement for this prototype scheme. That is, design a distance metric which can integrate color information into existing tracing algorithms or clustering methods so that it is able to seperate neighboring neurons assigned to similar colors. By completing the possible improvements, we are looking forward to establishing a more robust segmentation/tracing scheme for Brainbow/Flybow imagery in the future.

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REFERENCES

Bas, E. and Erdogmus, D. (2010). Piecewise linear cylinder models for 3-dimensional axon segmentation in brainbow imagery. In *Proceedings of ISBI*, 1297–1300.

Bier, E. (2005). Drosophila, the golden bug, emerges as a tool for human genetics. *Nature Rev. Genet.*, 6:9–23.

Gonzalez, R. C. and Woods, R. E. (2007). *Digital image processing*. Prentice Hall, 3rd edition.

Hadjieconomou, D., Rotkopf, S., Alexandre, C., Bell, D., Dickson, B., and Salecker, I. (2011). Flybow: genetic multicolor cell labeling for neural circuit analysis in drosophila melanogaster. *Nature Methods*, 8:260–266.

Lee, P. C., Chang, H. M., Lin, C. Y., Chiang, A. S., and Ching, Y. T. (2009). Constructing neuronal structure from 3D confocal microscopic images. *Journal of Medical and Biological Engineering*, 29:1–6.

Livet, J., Weissman, T. A., Kang, H., Draft, R. W., Lu, J., Bennis, R. A., Sanes, J. R. , and Lichtman, J. W. (2007). Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature*, 450:56–62.

Mathwork. http://www.mathworks.com/help/toolbox/images/f18-16264.html

Peng, H., Long, F., and Myers, G. (2011). Automatic 3D neuron tracing using all-path pruning. In *Proceedings of Bioinformatics [ISMB/ECCB]*, 239–247.

Rodriguez, A., Ehlenberger, D., Hof, P., and Wearne, S. (2011). Three-dimensional neuron tracing by voxel scooping. *Journal of Neuroscience Methods*, 184:169–175.