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 travels, encodes, decodes, and computes 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 ideal 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, Aequorin 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...
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 segmentation 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 operations.
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 between 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 skelennized image stack that would be a suitable input for tracing 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
4 EXPERIMENT RESULT

The source image stack we used consists of 131 image slices of dimension $1024 \times 1024$, and the sampling resolutions along x-, y- and z-direction are respectively 0.35, 0.35 and 1.0 $\mu$m. 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.

![Figure 4: Different views of two independent neuron cells and the fibers thereof.](image)

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 separated according to their color information.

![Figure 5: Different views of other two neurons.](image)

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 until 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 separate 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


