

QTIP: Quantifier for Topographic Index of Presynaptic-terminal using 3D Confocal Imaging

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Abstract—Topography mapping is an important and general strategy for the nervous system to encode the locations of sensory stimuli. It has been difficult to analyze the relative spatial position between adjacent neurons in the same nervous system because of complex fine branches of axon terminals, especially at single-cell resolution. This paper describes an automatic image analysis tool QTIP (Quantifier for Topographic Index of Presynaptic-terminal), which quantifies fine-scale 3D structures’ spatial relative topography. Using confocal microscopic imaging assisted with advanced immunostaining, the presented image quantification tool enables automatic analysis of fine scale topography mapping in a 3D context, exemplified by the axon terminal topography analysis in *Drosophila* larva somatosensory system. The tool QTIP is developed as an ImageJ plugin and available at <http://faculty.cs.niu.edu/~zhou/tool/qtip/>.

Index Terms— Topographic mapping, 3D confocal images, presynaptic terminal, *Drosophila* larva c4da neurons, 3D object detection, robust adaptive threshold selection, ventral nerve cord

I. INTRODUCTION

A topography map, in the context of neuroanatomy, refers to the spatial pattern of ordered projections in the central nervous system that encodes features of stimuli. Topographic mapping is a general strategy to encode the locations of the sensory input and has been known to be fundamental to sensory processing of mammals [1]. The topographic maps of human sensory cortical homunculus [2] is an example of such map, which is the physical representations of the sensory anatomical parts of the body.

While many studies have been performed in vertebrates for topographic maps and their plasticity [3], the molecular mechanism underlying activity-dependent regulation of fine-scale topography is not well understood. The molecular analysis of fine-scale topography requires genetic manipulation and the reliable identification of the topographic locations of the presynaptic terminals, both at single-cell resolution *in vivo*. *Drosophila melanogaster* is a model system that can potentially meet such requirements. Particularly, confocal microscopic

imaging combined with genetic mosaic clones are now able to provide three dimensional (3D) images of fine scale topographic locations, such as those of presynaptic terminals of *Drosophila* larva at single-cell resolution. These techniques are important for the studies to understand the molecular mechanism underlying the activity-dependent regulation of topographic mapping. Such study of fine-scale topographic mapping using 3D confocal image of *Drosophila* larva is the focus of this paper. From the perspective of bioimage informatics, the task is the reliable identification of the locations of the presynaptic terminals, as well as a robust quantification of their relative spatial order. The experimental aspect of the research including the immune labeling and genetic mosaic clones were reported in [4]. In this paper, we focus on the computational modules that automatically quantify the afferent terminals’ spatial relative topography in the *Drosophila* larva central nervous system.

Specifically, we present an automatic image analysis tool, named QTIP (Quantifier for Topographic Index of Presynaptic Terminals). To study the fine-scale topography in *Drosophila* somatosensory system, QTIP identifies presynaptic axon terminals using 3D confocal images of three nociceptive neurons in *Drosophila* larva (which are termed the class IV dendritic arborization or *C4da* neurons), then quantifies their fine scale spatial relationship in the *Drosophila* larva ventral nerve cord (VNC).

We will explain the algorithms employed by QTIP that quantify the fine structures, as well as the quantification of relative spatial order in the section of Methods, then report the developed software followed by exemplary experimental results as well as conclusions.

II. METHOD

The topography map of three *C4da* neurons in *Drosophila* larva is studied, which are the dorsal neuron ddaC (D), middle neuron v’ada (M), and ventral neuron vdaB (V). The dendrites of these neurons tile the larval body wall as an array of detectors for noxious stimuli, and respond to heat, harsh mechanical and intense light [5]. The presynaptic terminals of these three *C4da* neurons in a hemi-segment are confined in a compact, synapses-

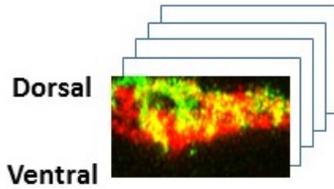


Figure 1. An image stack of the axon terminal of a *Drosophila* nociceptive neuron (green) and the c4da neuropil (red). Shown is the maximum intensity projection of all stacks. The image is enhanced for better visualization.

enriched neuropil, termed *C4da neuropil*. In the context of this paper, C4da neuropil is referred as the area where synaptic connections reside at the center of the VNC.

To examine the spatial map of the C4da presynaptic terminals, images were collected as three dimensional stacks using a Leica SP5 confocal system (Leica Microsystems) equipped with a 63X oil lens (Leica, Plan-Apochromat, NA = 1.4). The image stacks were deconvolved and rotated so that the slices are parallel to the A-P (anterior-posterior) axis and D-V (dorsal-ventral) axis of the VNC. Figure 1 shows an example of such image stack with the red channel and the green channel being the neuropil and axon terminal clone, respectively. For the example image, mosaic analysis with a repressible cell marker (MARCM) [6] was used to locate the synaptic terminals of single C4da neurons in the C4da neuropil.

Given the image stacks of immunostained neuropil and axon terminal, the computational goal is twofold: 1) to automatically detect the presynaptic terminal of the neuron of interest in the multi-channel image stack; and 2) to quantify the relative topographic position of an axon terminal clone in the neuropil, for revealing the spatial order of the three neurons under study.

The task presents several computational challenges: First, the axon terminal has fine-scale 3-dimensional tree-like structure. As a result, using 2D analysis would be insufficient since a 2D projection would blur the intricacy of the fine scale structure and compromise the quantification of relative position among different afferent terminals. Second, due to the fine size of the structure and the nature of the staining experiment, the images are of low contrast and often dim. It increases the difficulty in extracting the structures in the channels. Third, the images contain staining noise thus denoising is important for effective quantification.

In following subsections, we will describe the two aspects of our task, namely presynaptic terminal detection and topography quantification. Workflows designed for such fine-scale analysis assisted by suitable topographic measure are employed to address the related challenges.

2.1. Detection of the presynaptic terminal

Figure 2 shows the flow of the major algorithms in QTIP. As we can see from Figure 2, employed algorithms to detect the axon terminals include several denoising strategies applied at different stages, and iterative image enhancement, as well as robust image segmentation for both channels of neuropil and axon clone. The detected objects are then subject to spatial map quantification to be described later.

A. Territory-based Denoising using Two Channel Validation

Confocal image stacks from immunostaining subject to staining artifacts. After despeckling, we perform territory-based denoising, which is based on the territory of neuropil: By definition, presynaptic axon terminals of the C4da neurons are confined within the compact, synapses-enriched C4da neuropil. We thus can use two channel cross-validation to computationally cleanup noise for the axon terminal channel that are outside of the expected region. Specifically, territory-based denoising is performed using the following steps:

- 1) Combine the signal of the axon and neuropil channel to strengthen the signal for territory analysis.
- 2) Perform maximum intensity projection in the z direction to obtain the territory mask.
- 3) Calculate the signal territory boundary on dorsal and ventral sides.
- 4) Denoise the axon terminal channel and the neuropil channel using the territory mask by removing the signals outside of the mask boundary.

B. Iterative Enhancement and Object-based Denoising

The neuropil channel is typically dim. Image enhancement is essential for the subsequent signal detection. Enhancement based on histogram equalization, including advanced ones such as CLAHE [7], has been found less effective for our purpose because it is brings out background noise in the images. Instead, we employ iterative histogram stretching after denoising based on territory is performed. We do so by gradually stretching the pixels of lower intensity to the span up to 255. The intensity at iteration $t+1$ and location (x,y,z) is adjusted as following:

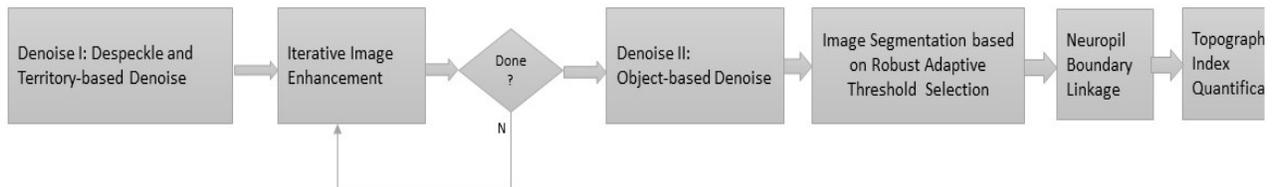


Figure 2. Algorithm flow of the QTIP.

$$f(x, y, z, t + 1) = \frac{f(x, y, z, t) - f_{min}}{f_{max}(t) - f_{min}} * 255$$

where $f_{max}(t) = 255 - t * interval$, with *interval* set to 10, and f_{min} set to 0.

The iterations stop when the average intensity in the neuropil channel reaches a user-specified threshold, e.g. 80, or it fails to reach the desired intensity within reasonable limit in which case the image will not be used for topography quantification.

After a round of median filtering, denoising based on object morphology is further applied to the enhanced neuropil channel. Specifically, 3D object detection based on 26-neighborhood 3D connectivity analysis [8] is performed to remove small non-connected objects. Objects that are smaller than 5 voxels and separated are considered as artifacts and removed.

C. Segmentation using Robust Adaptive Threshold Selection

For effective image segmentation, QTIP uses Robust Adaptive Threshold Selection (RATS) [9] to extract the axon terminal and neuropil foregrounds. RATS makes use of a hierarchical quadtree structure to adapt to the local contrast changes. Combining gradient and intensity information, RATS is able to capture the local thresholds in the small regions which are then bilinearly interpolated to the entire image. In QTIP, the step of RATS-based segmentation results in the extracted axon terminal and neuropil from their respective channel, ready for fine-scale spatial relationship analysis.

2.2 Quantification of Relative Spatial Relationship

2.2.1 Topographic Index

To quantify the relative topographic position of the clone in the neuropil, we have defined the measure Topographic Index (TI) [4] for measuring the position of a presynaptic terminal in neuropil. TI is calculated based on the position of each voxel of the clone image stack as below:

$$TI(x, y, z) = \frac{y_d(x,z) - y}{y_d(x,z) - y_v(x,z)} \quad (1)$$

which (x,y,z) is the location of a foreground axon voxel, y_d and y_v are the y locations of the neuropil's dorsal and ventral boundaries. Y coordinate represents the Dorsal-Ventral axis and Z axis is the Anterior-Posterior axis.

The overall topographic index of a clone is the averaged sum calculated by

$$TI = \frac{\sum_{x,y,z} TI(x,y,z)}{n} \quad (2)$$

where n is the total number of clone voxels in the 3D image stack.

TI was defined to serve as a quantitative measure of relative spatial relationship by considering the 3D structure of the axon terminal. By definition, TI is between 0 and 1. The smaller the value, the closer an axon terminal is to the dorsal side of the neuropil.

2.2.2 Boundary Linkage and Quantification

As seen from Equations (1) and (2), in order to calculate TI, dorsal and ventral boundaries of the neuropil need to be obtained for the 3D image stack. We perform boundary linkage slice by slice. For each slice, we find the dorsal and ventral boundary in the neuropil channel by locating the uppermost and lowermost voxel in the y direction. If a gap presents for a given (x, z) due to the branchy and dim nature of the signal, then the two nearest boundary points are linked to fill the gap. This yields the y_d and y_v for each (x,z) in the image stack, as needed by Equation (1). The result can also be understood as an efficiently calculated 3D contour of the neuropil. TI of the axon terminal for the given neuron can then be calculated accordingly using Equation (2). In addition, several other morphology metrics are also reported by QTIP such as volume ratio of the clone and neuropil signals which will be described in next section.

III. SOFTWARE AND RESULTS

QTIP is developed as an ImageJ plugin for ImageJ 1.44 or later. It is available for download at <http://faculty.cs.niu.edu/~zhou/tool/qtip/>.

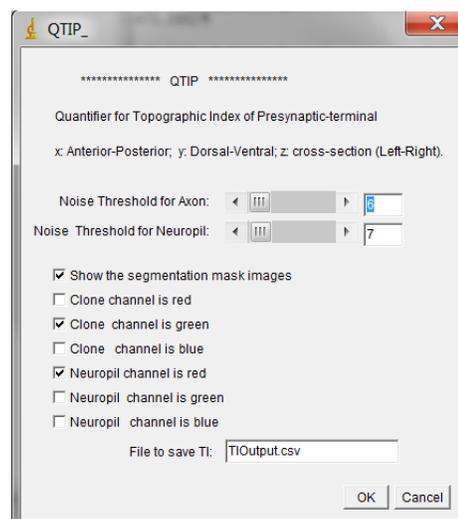


Figure 3. User interface of QTIP.

QTIP provides automatic fine scale 3D quantification for relative positions of a single neuron's axon terminals. It takes 3D images containing clone and neuropil channels, and outputs four quantifications including TI, all valued between 0 and 1:

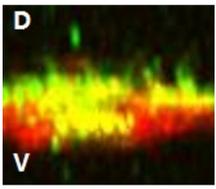
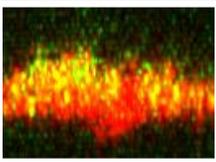
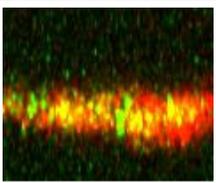
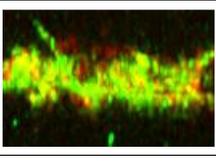
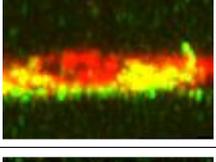
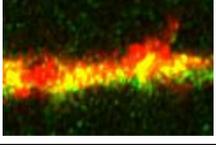
- Topographic Index (TI) as explained above.
- Volume Ratio: The ratio between the volumes of the clone and neuropil.
- Dorsal boundary position: The average position of all dorsal boundary voxels. The smaller the value, the closer is the axon's dorsal boundary to the neuropil's dorsal side.
- Ventral boundary position: The average position of all ventral boundary voxels. The smaller the value, the closer

is the axon's ventral boundary to the neuropil's dorsal side.

QTIP can be run in both single stack mode and batch process mode, with the latter suitable for high throughput analysis. Figure 3 shows a GUI of the system. The major parameters are the thresholds used by RATS segmentation. User manual is also available on the software website.

Table 1 exemplifies results obtained by QTIP on relative positions of axon terminals of three adjacent nociceptive C4da neurons (ddaC, v'ada and vdaB) in third instar *Drosophila* larvae.

Table 1. Topographic indices and volume ratios quantified by QTIP for different types of C4da neurons, with the green channel being the axon terminal clone and the red channel being the neuropil. Two examples are included for each type. Images are maximum intensity projection of the 3D image stack. The Y-axis is the dorsal-ventral (D-V) axis. A smaller TI indicates that the axon terminal is closer to the dorsal side of the neuropil.

Neuron	Image (MIP)	Topographic Index	Volume Ratio
ddaC		0.45	0.60
ddaC		0.36	0.15
v'ada		0.49	0.06
v'ada		0.52	0.51
vdaB		0.65	0.45
vdaB		0.61	0.40

From the examples shown in Table 1, we observe that for ddaC neurons, the TIs are the smallest and typically less than 0.5, which indicate that the ddaC axon terminals are located closer to the dorsal side in the neuropil. For the two v'ada neurons, their TIs are about 0.5 despite that they have very different volume ratios, which means that spatially they are located around the center of the neuropil. For vdaB neurons, the TIs are the largest, which indicate that the vdaB neurons are the closest to the ventral side.

These results suggest that, in *Drosophila* early 3rd instar larvae, the topography map of C4da presynaptic terminals of ddaC, v'ada and vdaB neurons are arranged in a dorsal to ventral map, in a way consistent with the dendritic field coverage. It is also worth pointing out that, by providing a quantitative measure of topographic index and other related output, QTIP enables the analysis of the plasticity and refinement of topographic map under molecular cues [4].

IV CONCLUSION

The paper describes an image analysis system QTIP that automatically quantifies the fine scale relative relationship using 3D confocal images, which has been instrumental in the analysis of topography mapping in a nervous system. With the increased availability of confocal image quantification, we expect the system and the algorithms employed in QTIP to have applicability in quantifying relative spatial positions of fine scale cellular or subcellular structures in a 3D context.

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