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Brainbow Image Segmentation Using Bayesian Sequential Partitioning

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Abstract—This paper proposes a data-driven, biology-inspired neural segmentation method of 3D drosophila Brainbow images. We use Bayesian Sequential Partitioning algorithm for probabilistic modeling, which can be used to detect somas and to eliminate crosstalk effects. This work attempts to develop an automatic methodology for neuron image segmentation, which nowadays still lacks a complete solution due to the complexity of the image. The proposed method does not need any predetermined, risk-prone thresholds, since biological information is inherently included inside the image processing procedure. Therefore, it is less sensitive to variations in neuron morphology; meanwhile, its flexibility would be beneficial for tracing the intertwining structure of neurons.

Keywords—Brainbow, 3D imaging, image segmentation, neuron morphology, biological data mining, non-parametric learning.

I. Introduction

IT is amazing that how little we know about our brain. Occupied by numerous neurons interweaving the whole structure, the brain processes enormous information every day and is prompted to react to any environmental stimulation anytime. Although scientists have recognized some perceptions are related to certain sections inside the brain, a thorough understanding of how neurons communicate and transmit information has not yet been completed. The scientific study of neuroscience aims at unveiling those secrets inside the brain. Neuroscience is a multidiscipline that requires high level of integration. With advances in molecular biology, electrophysiology, and bioinformatics, neuroscience has enjoyed fruitful discoveries for the latest centuries. However, one of the biggest challenges remains to map all the neural networks inside such a delicate structure onto a comprehensive system, the connectome. To study the fabricate structures in the brain, many methods have been proposed and utilized [19]; however, they all have their limitations. One of the most common approaches is to stain proteins contained in the neuron. This technique can be dated back to late nineteen century: Camillo Golgi first developed a staining procedure that could show one or more neurons in monochrome. This method was later adopted by Santiago Ramon y Cajal. Both of them became Nobel Laureates in Physiology or Medicine in 1906. Starting from that time, neuroscientists were equipped with a much more powerful tool than ever for observing and describing neurons in the brain. Many brain-related deceases were able to find causes and treatments. However, the thorough understanding of neurons was still very tough due to comparatively little information extracted per experiment. Think of comparing that information with the scale of the brain: for example, it is estimated that a human brain has roughly 100 billion neurons; even a tiny fly has approximately 100 thousands of neurons. As a result, monochromic staining was very inefficient if neuroscientists would have to use that to map all neurons; nor was it accurate enough to distinguish neighboring neurons if neurons were stained simultaneously. As for image processing, many automatic neuron-tracing algorithms had been proposed [1]-[5]. Nevertheless, all those methods were dedicated for monochromic images back in old days. It was the limitation of biological experiment and insufficiency of image information that made the development of connectome staggered.

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However, the scientific needs for understanding the brain never halt. In 2007, a major breakthrough in neuron-imaging technique, Brainbow, was invented. Developed by a team led by Jeff W. Lichtman and Joshua R. Sanes [6], Brainbow shed new light on connectomics. Nominated by its fluorescent nature, Brainbow allows not only flagging more than 100 different neurons simultaneously but also distinguishing neighboring neurons by different colors, almost solving previous shortages all at once. However, it raised complexity for image processing, especially neuron segmentation. For one reason, snaking neurons cause serious crosstalks if the image resolution is not high enough. Other properties, like the saturation of fluorescence, also make image processing difficult to handle. Up to present, there has not been any complete solution for automatic multicolored-neuron-image segmentation. Thus for quite a long time, neuroscientists had to trace neurons by eye to get reliable segmentations. It is extremely labor-intensive and time-consuming. In 2013, Shin-ya Takemura et al. [7] proposed a method of constructing a visual motion detection circuit, in which a way of processing the neural image is also involved. However, it still requires human interaction. In this paper, a new method is proposed to treat the three-dimensional structure as a whole, adaptively eliminating and amending for undesirable image deficiencies, such as crosstalks and insufficient resolutions.

II. Related Works

Early works as [8], Cohen et al. showed that it is possible to design automatic cell tracking procedures. Later, several works were able to trace monochromic neuron images [1]-[5]. By selecting proper filtering criteria, some fabricate structures can be extracted. But they all suffer from the danger of losing important data, because, in a neural image, signal intensity might not be correlated with its significance.

On the other hand, few works were dedicated for Brainbow images, since it is still developing and also complex to process. First, because of the fluorescent effect, some areas with stronger fluorescence would saturate, therefore staining or even shadowing its neighboring neurons. Wu et al. [9] proposed a way of probabilistic fitting to eliminate such crosstalk effects. We will use the idea but with a new estimation method for the probability model. Second, some works attempted to model the shape of neuron cells, based on which Bas et al. [10] proposed a cylinder-shape model to delineate the structure of neurons. However, due to the irregular outlines of neuron fibers, it is difficult to assign a general shape to model them. Moreover, the ends of dendrites and axons are usually places where transmissions of cerebral signals occur; even a dim voxel might serve as important information. Therefore, treating each voxel as precious information as they should be, this perspective taken on [3] was dedicated for resolving this issue. In this work, this idea is extended to multicolor three-dimensional images.

For a brief summary, the proposed method is voxel-perspective, automatic and also adaptive for tracing and segmenting all neurons in a Brainbow dataset. We consider the 3D image as a whole, and also try to mend the lower resolution in the third dimension. Therefore, unlike methods proposed in [7], whose segmentation was done by segmenting 2D images first and then stitching back to 3D, the proposed method can prevent the segmentation task from losing information along the third dimension. Also, it does not need any predetermined thresholds, as the spectral matting methods proposed in [9] and [11], nor does it require the object being traced to have any specific morphology. It is less sensitive to variations in the snaking structure; meanwhile, its flexibility would be an advantage for tracing intertwining structures.

The organization of this paper is as follows. The Brainbow imaging technique and its difficulties of image processing will be introduced in Section III. Section IV is the complete flow of the proposed method with details described. Section V will show the experiment results of using the methodology for Brainbow image segmentation. Finally, Section VI is the conclusion.

III. The Brainbow Image

The Brainbow image is acquired by confocal microscopes. Confocal microscopy is a laser scanning technique which allows the recording of three-dimensional images of small objects stained with a fluorescent dye. It is one of the most common sources for neuron digitalization nowadays. During scanning, each voxel is illuminated in turn by a focused laser beam. The photons emitted by the fluorescent dye are filtered by a small pinhole, and the remaining photons are detected by a photo multiplier. The captured image would later be stored into a sequence of two-dimensional image stacks, forming a three-dimensional image set [19]. The image format we acquire is LSM, which is an exclusive format of Carl Zeiss AIM Inc. There are three to four channels for each two-dimensional image, and the intensity is from 0 to 255. Fig. 1 is an example of such an image, and Fig. 2 is the partial zoom-in of Fig. 1.

Our goal is to segment neuron paths automatically in any given Brainbow image. And the main challenges are: First, it can be seen clearly from Fig. 2 that the resolution is not enough for distinguishing signals from noises; in addition, many voxels contain simultaneously more than two neurons. Second, still seen from Fig. 2, there are many isolated fluorescent dots distributing randomly on the image. They may be isolated due to low resolution while they should be connected, or they may simply be noises. The criteria for seperating these two should be examined carefully. Third, crosstalks between channels also happen randomly. Not only have we to detect them but also to amend deficiencies due to crosstalks. Combined with additional biological morphology of the neuron, the proposed method can be used to achieve automatic neuron segmentation effectively.

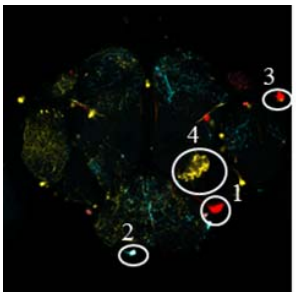


Fig. 1 A 2D slide of a 3D Brainbow image set with several somas circled

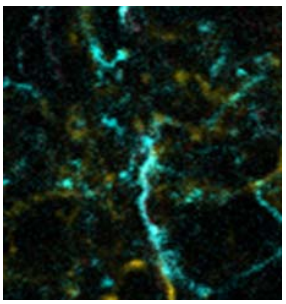


Fig. 2 Partial zoom-in of Fig. 1

IV. Methods

Fig. 3 shows the flow of the proposed methodology. First the Bainbow image dataset is acquired and filtered out noises. Because confocal laser scanning of multicolor stacks is generally done at speeds of about 1 us per pixel or less to save time, the small number of photons collected for each pixel gives rise to sufficient shot noise to cause perceptible local color differences. After denoising, several features are extracted: (1) coordinates of voxels, and (2) color information of those, namely their RGB values. Each voxel contains five dimensions of properties, and then the Bayesian sequential partitioning (abbrev. BSP [18]) is used to

detect somas. The returned probability density is an indicator for regions containing somas. After soma detection, we start to trace dendrites and axons. Here an adaptive inspection window is imposed in this algorithm. The main function of the inspection window is to detect the direction of growth and filter voxels inside. It is an imaginary 3D box that encloses the neuron under tracing. In each inspection window, two stages of filtering are done: connecting filtering and color filtering. Then we use its direction of growth as an indicator for enlarging the inspection window. Every time after color filtering, we check whether the neuron still has the tendency to grow. If it is true, we iterate this procedure until finding no tendency of growing. More details will be provided as follows.

A. Soma Detection

Somas are those areas that have extremely strong brightness intensity and the only round, dense structure in the whole image. As Fig. 1 shows, circles 1 to 3 are somas but circle 4 is not. They are obvious to human visual perception and can be easily acquired by image erosion, as proposed in [11]. However, we here propose a different approach, which utilizes the BSP to process all voxel data first and retrieve their joint probability density as indicators for soma locations.

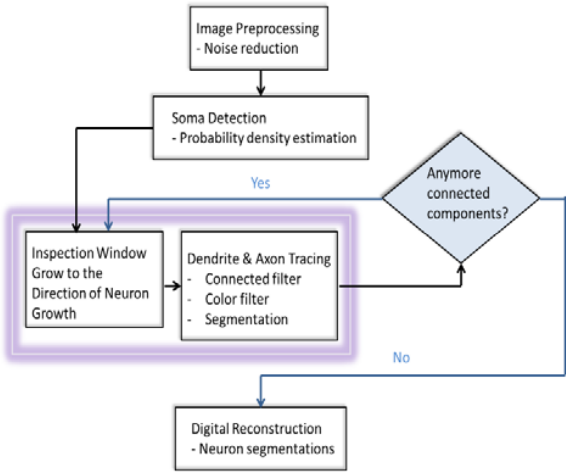


Fig. 3 Flow of Brainbow image processing with BSP

We define each voxel V_i as the function of its own coordinates (in 3-dimensional Cartesian coordination) x, y, z, and its r_R and r_G, which are defined as

$$r_R = \frac{R_R}{R_R + R_G + R_B} \tag{1}$$

$$r_G = \frac{R_G}{R_R + R_G + R_B} \tag{2}$$

$$r_B = \frac{R_B}{R_R + R_G + R_B} \tag{3}$$

where R_R, R_G, and R_B are the R, G, and B intensities correspondingly.

Since Brainbow image was acquired by confocal microscopy, its RGB intensity of a certain fluorescence color varies inherently but the proportion of its color ingredients rarely changes. In other words, the intensity ratio of R:G:B is comparatively more stable throughout a complete neuron, rather than the original R, G and B intensities. Since r_B is completely dependent on r_R and r_G, here only r_R and r_G are included as parameters. After obtaining that information, we use the BSP to find their joint probability density $P(X = x_i, Y = y_i, Z = z_i, R_R = r_{Ri}, R_G = r_{Gi}|D)$. The BSP will perform variable selection, which adjusts the input data and returns the most suitable bin width of a histogram, so that those bin partitions with high probability density are most likely to be regions that actually contain somas.

Generally, there are two kinds of situations for a partition to possess a high probability density. One is that the partition contains soma. The other is that the partition contains synapses. They can be seperated by their different morphology: somas have much higher density, defined as voxels per volume, than synapses do. By applying this criterion to partitions possessing high probability density, we can identify somas out.

B. Connected Filtering

From those somas found as starting points, the next step is to trace rest of the neurons from them. Starting from a soma SOM_i, we use (1) connected component filter C and (2) color ratio filter R to determine the rest of voxels v_r such that for every neuron

$$N_i = SOM_i \cup v_r. \tag{4}$$

Since obviously v_r should be connected with the soma, connected component filter is adopted first. An inspection window is created to save all six-direction boundaries of the neuron: minimum x, maximum x, minimum y, maximum y, minimum z and maximum z. The inspection window encloses the neuron under tracing. In each iteration, only voxels inside one inspection window are taken into consideration, preventing waste of repetitive computation and memory occupation. Besides, the inspection window has another function, which is to detect where the neuron grows. By detecting whether any side of the inspection window has neuron voxels laid on, we can further decide to where the next inspection window should grow. We record those sides with voxels laying on as S_i, and those voxels as V_S .

Then we start to filter inside the inspection window. Firstly, binary connected filters are used . A threshold of brightness is set to separate signal and noise. We define $I(V_i)$ as an indicator for signal and $B(V_i)$ as the brightness of voxel V_i. Therefore,

$$I(V_i) = \begin{cases} 0, & B(V_i) < th \\ 1, & B(V_i) \geq th. \end{cases} \tag{5}$$

If $I(V_i)$ equals one for V_i, we treat V_i as a real signal; else, it is a background noise. Each time as the inspection window grows, the connected component C_i is obtained by

$$C_i = C_{i-1} \cup (C_i \cap V_S). \quad (6)$$

That means, given the soma SOM_i as C_1, every time the size of the inspection window increases, a new C_i is obtained. The union of the new C_i and its previous one, namely C_{i-1}, should represent the neuron segmentation been found so far. In addition, the intersection of C_i and V_S is taken first, because it is then ensured that the connected components found in the new inspection window are indeed connected with neurons from its previous inspection window.

The connected component filter has been well accepted as providing a rich, scale-invariant description for grey-level images. Its data structure can be efficiently implemented using a connected-component tree structure. It has been involved, in particular, in the development of attribute filtering [12], object identification [13], and image retrieval. In the context of segmentation or recognition tasks, it enables performing object detection without having to precompute a specific threshold (which is usually an error-prone task). It is highly suitable for fabricate structures whose contour contains important information. It scoops out wanted voxels without altering the rest of the image. Another advantage of connected-component tree structure lies in its low algorithmic cost: many efficient algorithms have been designed to compute it [14]-[16]. The achievable time complexity is $O(N + E)$, where N and E are number of nodes and edges respectively. Together with inspection window, which allows us processing only a small amount of voxels per iteration, the algorithm is highly efficient. Therefore, the proposed method suits handling of big data like the Brainbow, whose bulk volume adds to a surge of the data amount, comparing with a typical 2D image. Therefore, it is necessary to do such data reduction and focus on only data containing information of interest.

Fig. 5 shows one soma detected by BSP, and it is enclosed in the inspection window, which will detect the direction to which the neuron keeps growing. As the inspection window keeps being enlarged, the neuron region-grows to its connected voxels. After several iteration, Fig. 6 shows that the neuron has grown form its soma (inside the right box) to some extension of its dendrites. When more neural voxels are collected into the inspection window, the better control of the neuron under tracing is acquired. As a result, connecting criteria can be set adaptively in each iteration. For example, as we gather more voxels, we will know their brightness distribution over their distances from soma. Fig. 4 shows such a distribution of several neurons. If the th in (5) is chosen too high, important information might be missing as we trace to the end of dendrites and axons, whose brightness is much lower than the soma. On the contrary, if th is chosen too low, the danger is to involve too much noises that shall not be involved. As a result, the proposed method does not depend on a global th as an universal indicator; instead, it imbues the flexibility in each iteration. Each th, threshold of brightness, and δv, increment of volume of the inspection window, are calculated by Euler method [17]. That is, we predict th_{n+1}, the brightness threshold following th_n, by its rate of change over its current inspection windows volume v_n; that is,

$$th_{n+1} = th_n + \delta v \cdot f(th_n, v_n), \text{where} \quad (7)$$

$$f(th_n, v_n) = \frac{th_n}{v_n}. \quad (8)$$

Since the threshold of brightness and the size of the inspection window are chosen adaptively, it can adjust to neuron morphology (irregular shape) and image quality (nonhomogeneous brightness) according to its local information. Also, since each time we only calculate C_i and finally find the union of them as in (6), every iteration step is independent. Therefore, the iteration does not take up more time complexitythe time complexity is still dominated by connected component filtering.

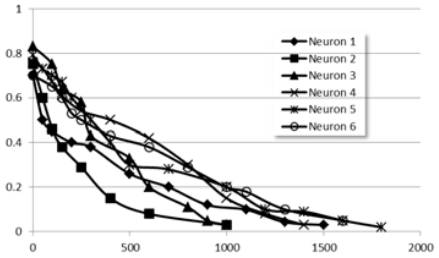


Fig. 4 Signal brightness (normalized from 0 to 1) vs. lattice distance from its own soma

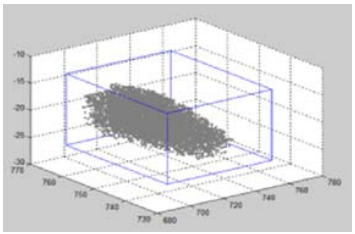


Fig. 5 A soma detected from BSP and its corresponding inspection window

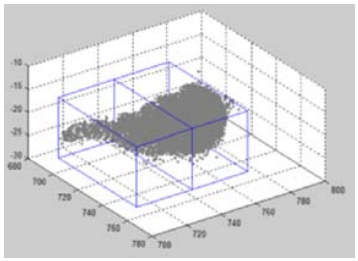


Fig. 6 Growing neuron from Fig. 5. undergoing the proposed segmentation method

C. Color Filtering

The second filtering stage is the color filter. There are two color filters: one for eliminating crosstalk effects and the other for selecting right colors and also amending for intersecting

areas whose colors are mixed and changed, prone to be filtered out after typical color filtering for a limited bandwidth.

Instead of setting criteria based on common color spaces like RGB, HSB, or HSV for filtering, we use the ratio of R:G:B defined in (1)-(3) as filtering criteria. Therefore, after partitioning and reconstructing, the following color patterns specifically to Brainbow are adopted in this methodology:

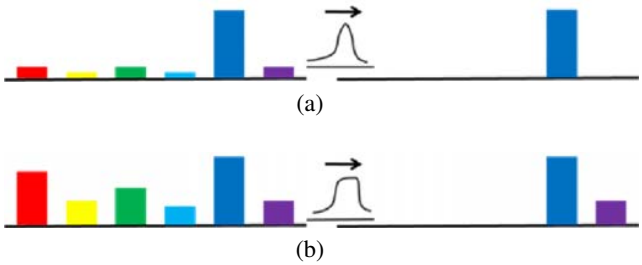
1) Color ratio stability: Throughout the whole neuron, its RGB and HSB/HSV value varies. What is kept steady is its ratio of R:G:B.

2) Stochastic color mixing: Generally, the color variation in hue changes gradually. However, in some areas where two or more neurons meet, their color might mix with each other because of the color-mixing effect of confocal microscopy recording. In those areas, it is expected that a sharp peak of change in hue value will happen.

To deal with these properties, first we use the color ratio (1)-(3) as an aid to filtering. Then, while growing the neuron, we will stop at each iteration to check the color ingredients of the current inspection window and adjust the color filtering criteria.

Since each color obtained from the confocal microscopy is a combination of three to four discrete color channels, the probability density function of each color component can be constructed. Indeed the BSP used earlier in soma detection also provides the information here. From BSP we obtain all the marginal probability density of each channel and their joint probability density. And this is the reason why we do not use image erosion for soma detection. Then we can use the probability density function of each channel as a color filter. After that, crosstalk effects among channels can be greatly eliminated [9].

The concept of the second color filter is illustrated by Fig. 7. Every time we come to a new inspection window, we analyze its color ingredients. For example, in Fig. 7(a), we see that since the majority color ingredient is blue, we should filter out all the other colors except blue. Fig. 7(b) shows another case. Now other than blue, a comparatively large amount of red components are involved in. Since magenta is the combination of blue and red, it will appear at areas where red and blue neurons meet. As a result, magenta should be preserved when tracing both red and blue neurons.



(a)

(b)

Fig. 7 An example of the function of the second color filter

V. Experiment Results

Fig. 8 shows the tracing process of a neuron under our proposed method, and Fig. 9 is a top-viewed, flatten complete neuron.

First, in Fig. 8(a) we start from a soma, which was detected by the soma detection method. Then we impose an inspection window on it and detect its direction of growth. Every time we detect its direction of growth, we enlarge the inspection window in that direction, filter voxels inside, and find unions of newly-found neuron paths with union paths from previous inspection window. Fig. 8(a) to (d) show the concrete tracing process of the methodology discussed in Section IV. Finally, Fig. 9 is complete neuron segmentation.

Since tiny voxels may represent important information for connectome, we have to treat them voxel by voxel. To allow flexible parameter selection according to local information of the neuron data, we use the concept of a growing inspection window to deal with voxels locally. Fig. 8(c) and (d) show the color filtering stage using local color information. Therefore, this methodology does not depend on any predetermined thresholds. It is a completely data-driven work that devotes to large data mining of biological images.

VI. Conclusion

Connectomics is now a major topic in science. Brainbow serves an innovative technique in neuron imaging, whose ultimate interests devote to map and understand the whole connectivity and functions of cerebral neurons. However, due to the complexity of dealing with the intrinsic stochastic property of Brainbow images whose details can not be carelessly ignored, it still lacks completely automatic ways of neuron segmentation, making the progress of new discovery hampered.

In this paper, we propose a new solution for automatic Brainbow segmentation by adaptively tracing neurons by their biological morphology. It not only saves computation time by reducing unnecessary data, but preserves important biological information. The experiment results show good accordance with some present known neuroscience. Another advantage is its flexibility: it can adapt to variations of neuron morphology and image quality - not any predetermined thresholds are needed, nor is any model that might not be completely suitable for modeling the irregular morphology of neuron fibers required. Results indicate that the proposed method can successfully trace multiple axons in dense neighborhoods. Though there are still some limitations regarding to the resolution of the original Brainbow image, we hope this automatic segmentation method will help neuroscientists accelerate new discoveries in connectomics. In future works, we also hope to apply this method to more biomedical image processing and data mining, catalyzing more new discoveries.

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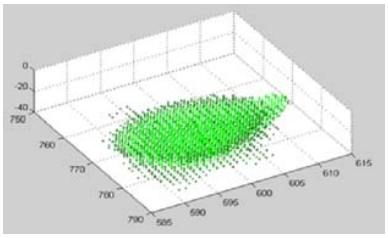
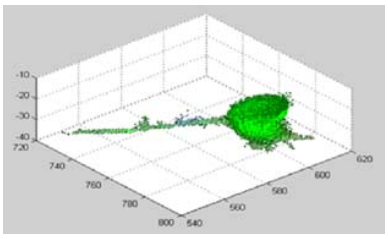
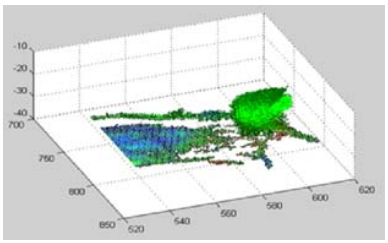


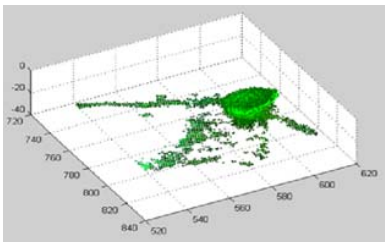
(a) Soma detected from BSP



(b) Growing from Fig. 8(a), the neuron grows in a particular direction



(c) As the neuron keeps growing, the connected-filter connects the neuron to some unwanted areas



(d) Filter out unwanted regions in Fig. 8(c) using proposed color filters.

Fig. 8 The tracing process of a neuron

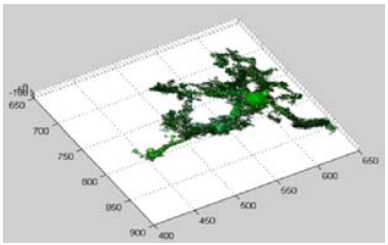


Fig. 9 A complete neuron extracted

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