Proceedings of the 2007 IEEE
International Conference on Robotics and Biomimetics
December 15 -18, 2007, Sanya, China

Compensation of binarized CLSM images for extraction of the form of a single neuron in the silkworm moth brain*

Kanako Nakajima and Taketoshi Mishima
Graduate school of Science & Engineering
Saitama University
255 Shimo-okubo, Sakura, Saitama, Saitama,
338-8570, Japan
{nakajima, takemi}@me.ics.saitama-u.ac.jp

Soichiro Morishita, Mihoko Otake and Hajime Asama
Research into Artifacts, Center for Engineering
The University of Tokyo
5-1-5 Kashiwanoha, Kashiwa, Chiba,
277-8568, Japan
{mori, otake, asama}@race.u-tokyo.ac.jp

Tomoki Kazawa and Ryohei Kanzaki
Research Center for Advanced Science and Technology
The University of Tokyo
4-6-1, Komaba, Meguro, Tokyo,
153-8904, Japan
{kazawa, kanzaki}@brain.imi.i.u-tokyo.ac.jp

***Abstract* - This paper proposes a method for automatic extraction of the form of a single neuron from image series which are captured with Confocal Laser Scanning Microscopic (CLSM). Deficiency parts appear in binary images through extraction process because of noises in CLSM images. It is needed manual operation to compensate it. Our method connects some discrete points picked up from the presumable area which exist neuron with smooth curves. Using this method, the form of a single neuron is represented by smooth curves, and it is reduced that the burden of manual extraction processes. We applied our method the task of extraction of the form of a pre motor neuron in order to verify availability of the method.**

Index Terms – the form of a neuron, binary images, deficiency parts compensation, Bezier curve

I. INTRODUCTION

It is known that the number of neurons of the insect brain is about $10^{5\text{-}6}$. It is far fewer than the mammalian brain which have about $10^{10\text{-}11}$ neurons [1]. Therefore the structure of the insect brain is comparatively simpler than the mammal's one, although they have the ability of learning and memory and behave intricately in adapting to circumstances. In other words, they have a good adaptability and they can control their behavior. Silkworm moths draw attention as a model animal, which is to understand a data processing from simulation to behavior. This is because it is relatively-easy that we analyze structure and mechanism of neural circuit in a brain of silkworm moths among a variety of insects. In addition, it is apparent that their behavior is formulaic against pheromone stimulation. For example, male silkworm moths exhibit a characteristic zigzagging behavior consisting of straight-line walking, zigzagging turns, and looping in response to sex-attractant pheromones released by their females [2][5]. The timing for shifting of the turning direction is synchronized to the sideways head movements. The flip-flop type responses synchronized with zigzagging behavior is recorded in pre motor neurons [5].

As above, one or small group of neurons in insect brain tends to be dominated the specific behavior or the function. Thus it is necessary to analyze circumstantially what neurons are related to the neuronal function and how generate them. Neuronal functions are strongly related to their form. Therefore it is very important to extract the form of a single neuron and construct a three-dimensional model of it in order to elucidate the structure and the mechanism of neural circuit. The model is constructed from cross-sectional image series of a single fluorescently-stained neuron. Those images are obtained by extracting of the fluorescently-stained region from the image series which are captured with Confocal Laser Scanning Microscope. Binarization is applied to extract them. Though, some deficiency parts may be observed through this process. It is a fatal issue in elucidation of structure. However this issue is difficult to avoid because it is brought by dyeing condition and autogenous fluorescence. Currently, the process is performed manually. Thus we need a large amount of labor and it is hard to process vast quantities of data. In addition, the result depends on each person's ability. We propose a method for automatic compensation of deficiency parts of the binary images through binarization process in order to extract the form of a single neuron.

II. THREE-DIMENSIONAL MODEL OF A SINGLE NEURON

A. Capture of the cross-sectional image series

A cross-sectional image series of a single neuron is needed in order to construct a three-dimensional model of a single

* This work has been partially supported by a Grant-in-Aid for Scientific Research on Priority Areas "Emergence of Adaptive Motor Function through Interaction between Body, Brain and Environment" from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

neuron. This image series is captured through the following steps [3].

1. Impale an intended single neuron with a glass microelectrode filled with a fluorescence dye.
2. Apply the 1-10nA electrical current to a glass electrode for injection the dye into the neuron.
3. Fix the brain in formaldehyde, dehydrate it with an ethanol series, and cleared it with methyl salicylate for get high S/N samples.
4. Capture the cross-sectional image series of a single neuron with Confocal Laser Scanning Microscope (CLSM).

In those steps, a central axis is not out of alignment, and we enable to capture high quality images. Fig. 1 is the silkworm moth brain. Fig. 2 shows appearance of injection of a single neuron in the silkworm brain. Fig. 3 is a projection image of the image series of pre motor neurons which are captured with CLSM.

B. Extraction of the form of a single neuron

Extracting of the fluorescently-stained region, we enable to construct a three-dimensional model of a single neuron. It is necessary to determine edge parts, branching parts and thickness of a neuron in order to elucidate structure of a neuron with the three-dimensional model.

1) Extraction of the form of a neuron with Single-Seed Distance Transform method

In previous works [4], firstly, they transform the image series into binary images, and they gain an image series of major dendrites of neurons.

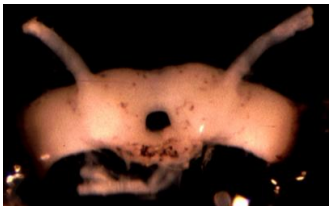


Fig. 1 The brain of silkworm moth

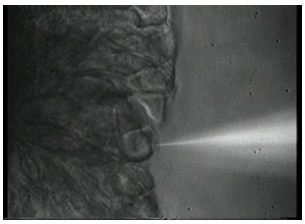


Fig. 2 Injection of single neuron

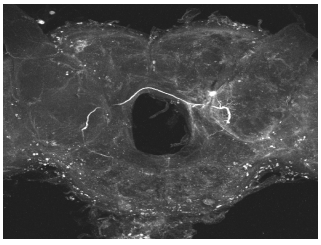


Fig. 3 A pre motor neuron which is captured with CLSM

Next, they transform the binary images into single-seed distance transform images with Single-Seed Distance Transform (SSDT) method. Fig. 4 and Fig. 5 show the schematic procedure of SSDT in the case that voxel A is a start voxel. This process operates in region which voxel value is not zero. Either automatically or manually, operation of SSDT is started by determining a start voxel, and setting its distance value to zero. SSDT operates by propagating distance values from a neighborhood voxel. For example, in Fig. 4, in the case of the voxel A has a distance value d, a distance value of a neighborhood voxel is $d + 1$. A distance value is increased by repeated application of SSDT, and propagation of distance value from start to end voxels as shown in Fig. 5. In Fig. 5, neighborhood voxels have the same distance value. This kind of group of voxels is called cluster. When a dendritic branch exists, two distinct clusters have same distance value. In Fig. 5, clusters that have $d = 5$, $d = 6$, $d = 7$ are it. In this case, the cluster with $d = 4$ is called the branching cluster. An edge cluster is defined as a cluster which has no neighborhood voxels and highest distance values. In Fig. 5, the cluster has $d = 7$ is it.

Lastly, the three-dimensional structure of the neuron is constructed with sequentially connecting clusters using their distance values.

Then a simulation of propagation of action potentials with this three-dimensional model is achieved [4].

2) Issue in extraction of form of a neuron from images capturing with CLSM

Using SSDT, it is possible to extract the form of a neuron, branching and edge clusters, when there are no deficiency

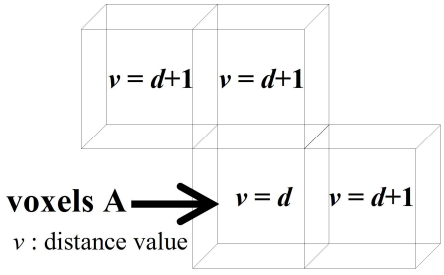


Fig. 4 Propagation of distance value next to voxel by SSDT

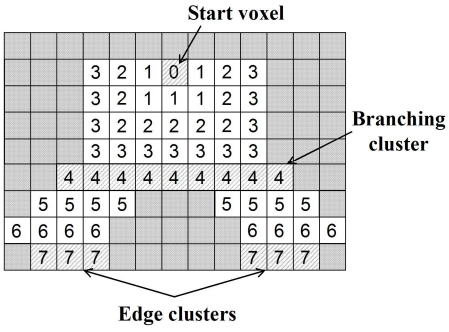


Fig. 5 An example of SSDT

parts in the image series. At the same time, the CLSM image series have a different of lightness caused by dyeing unevenness or optical noises. Some other tissues also may be stained. Although it is possible to clear away those noises using binarization process, deficiency parts appear in the image series as shown Fig. 6.

In those cases, it is difficult to determine whether a voxel is deficiency part or edge cluster, and we need to set a start voxel every time when the deficiency part is produced. Therefore the SSDT method is applied to an image series have no deficiency parts through binarization process. Though there are about 10^3 data in our database of CLSM images, most of them do not satisfy this condition which is necessary for SSDT. Thus it is often the case that we need to retouch the deficiency parts manually with a photo retouching software.

In previous works, methods for extraction of the form of a neuron are approached. However those methods are for images have no noises [6]. In addition, although method for compensation of deficiency parts, which is due to difference of image lightness, is approached, still it depends on manual procedure in some process [7]. Anyhow, those methods are just for extraction of a topological characteristic of neuron, and a thickness of neuron is not extracted. Generation parts and a transfer rate of action potential depend on the thickness of neuron [8]. Therefore the thickness of neuron is important information in order to elucidate structure of neurons.

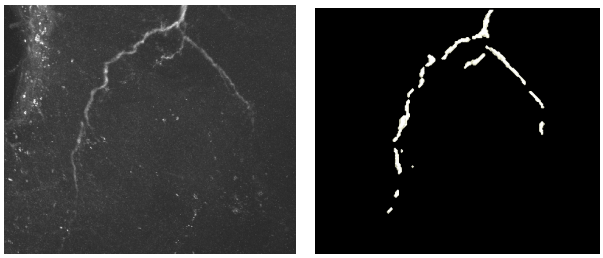
For this reason we propose the method which enables to automatically compensate the deficiency parts and extract the form of a neuron with involvement thickness of a neuron.

III. Proposed Method

A. Summary of proposed method

SSDT method is based on the premise that the image series have no deficiency parts; this means all voxel is coupled. In other words, if there were deficiency parts, voxels are not coupled. Then we consider the image series as not contiguous voxels but sets of discrete points, and connect those points with smooth curves. We explain this idea in Fig. 7. The binary images include no noises after binarization process as a prerequisite for proposed method.

Firstly, several points are selected from areas which are not background. The images are expressed in just with discrete points. This obtains similar sets of points regardless of with or without deficiency parts, when the gaps between selected points are larger than the size of deficiency parts.



(a) CLSM image (b) binarized image

Fig. 6 An example of deficiency parts through binarization process (a projection image)

Secondly, those points are grouped every branch, and they are connected with smooth curves. As the result of this operation, the deficiency parts are compensated.

Finally, thicknesses are interpolated each group. With this processes, the form of a single neuron is extracted from the CLSM image series. We describe detail of each procedure in the following sections.

B. A detail of proposed method

1) Selection of discrete points from a distance transform image

The procedure described in this section is to select discrete points from the binary images. We describe it using a two-dimensional model for the sake of simplicity, though actual extraction of the form of a neuron is performed with a three dimensional one.

Firstly, the binary images are transformed with distance transform. It is process to transform a pixel value of binary images into a Euclidean shortest distance from background pixels. Fig. 8 shows an example of a distance transform image.

Next, skeletons are extracted from the distance transform image. A skeleton is a central pixel of sphere when covering an image with sphere of necessary minimum. It is possible to reconstruct image perfectly using skeletons and the distance value of them. We consider distance values of skeletons as a thickness of neuron because it denotes inscribed diameter. Fig. 9 shows an example of extraction of skeletons from a distance transform image, and Fig. 10 is the result of an extraction of

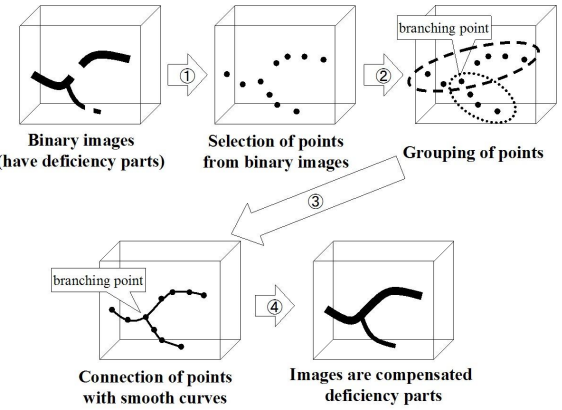


Fig. 7 The schematic diagram of proposed method

0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	1	1	1	1	1	0	0	0
0	0	0	1	2	2	2	2	2	1	0	0	0
0	0	0	1	2	3	3	3	2	1	0	0	0
0	0	0	1	2	2	2	2	2	1	0	0	0
0	0	1	$\sqrt{2}$	$\sqrt{2}$	1	1	1	$\sqrt{2}$	$\sqrt{2}$	1	0	0
0	1	$\sqrt{2}$	$\sqrt{2}$	1	0	0	0	1	$\sqrt{2}$	$\sqrt{2}$	1	0
1	$\sqrt{2}$	2	1	0	0	0	0	0	1	2	$\sqrt{2}$	1
0	1	1	1	0	0	0	0	0	1	1	1	0

Fig. 8 An example of distance transform image

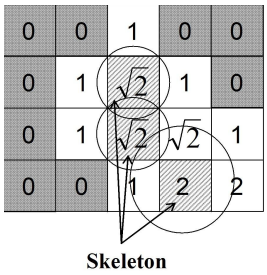


Fig. 9 Extraction of skeleton

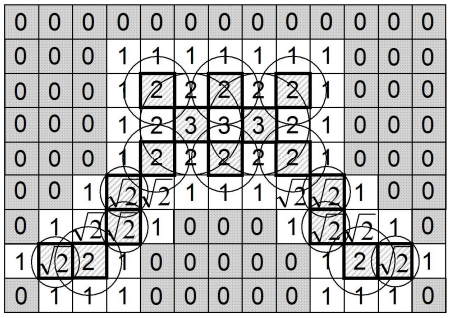


Fig. 10 Extraction of skeleton from Fig. 8

skeletons from Fig. 8.

Finally, several points are selected discretely from skeletons. In this paper, those points are selected in such a way that every gap should be uniformly. The gap is based on a maximum size of deficiency parts...

2) Connection of points with smooth curves

The procedure in this section is to connect the discrete points which are selected from skeleton with smooth curves.

Firstly, as shown Fig. 11, those points are grouped every branch because a neuron has many branch and a branch is a smooth curve. In first grouping, a start point set in an edge point. After first grouping, a start point set in a branching point. This makes it possible to connect between groups. A branching point is replaced a skeleton is proximity to a branching point which is extracted through thinning process. The points belong in the same group appear proximity each other, and the angle between a certain point and the neighbor one should be about π. For this reason, we employ a distance of two points and an angle made with three points as shown Fig. 12 as evaluation index of grouping. The angle is calculated as follows:

$$f(i,j,k) = -\cos(\angle p_i p_j p_k) = -\frac{\overrightarrow{p_j p_i} \cdot \overrightarrow{p_j p_k}}{\left\|\overrightarrow{p_j p_i}\right\| \left\|\overrightarrow{p_j p_k}\right\|}, \quad (i,j,k = 0,\cdots,n-1) \tag{1}$$

n is the number of points, and $i \neq j$ and $j \neq k$ and $k \neq i$. We consider that the distance of points in same group is smallest and the angle of it is bigger than threshold. In addition, if the distance was longer than d, the point does not belong in same group because distance of two points which belong in same group is not so long.

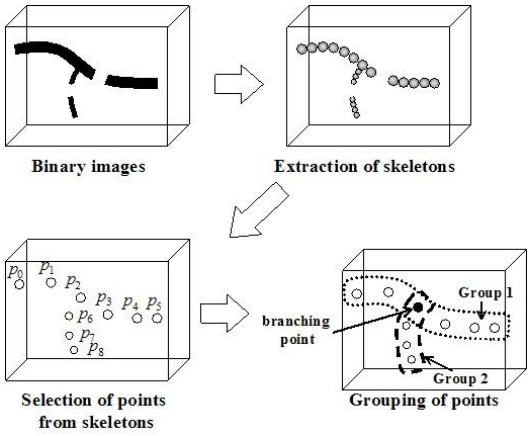


Fig. 11 Grouping of slate points

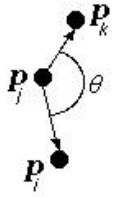


Fig. 12 Angle in (1)

Secondly, the points are connected every group with Bezier curves. Bezier curve is draw based on following equations:

$$R(t) = \sum_{i=0}^{n-1} p_i B_i^n(t) \tag{2}$$

$$B_i^n(t) = \binom{n}{i} t^i (1-t)^{n-i} \tag{3}$$

The reason which we use the connection method not passing by the points strictly is as follows. The points are not always being on center line because the points are selected from skeletons in proposed method. In the case of a neuron, this predisposition is remarkable because surface of a neuron is convexo-concave. Bezier curve is passing only a start point and an end point, and connects points with influence of other points to some extent.

This grouping and connection of points are processed sequentially.

3) Interpolating of distance value on Bezier curve

Deficiency parts are connected with above procedures. However it is nothing more than the extraction of a topological characteristic of a neuron. In this section, we describe a procedure for deciding of thickness of the curve.

The distance values of control points of Bezier curve are defined definitely because those points are picked up from skeletons. In the other hand, the values of other points on Bezier curve are not necessarily defined. Then distance values on Bezier curve should be interpolated with the distance

values of control points and surrounding skeletons of them as shown Fig. 13. In Fig. 13, p_0 is a start point and p_3 is an end point and d_0 is distance value of p_0.

Firstly, the distance value of p_1' which is the nearest point from p_1 on Bezier curve is decided. The distance value of p_1' is replaced with an average of distance values of skeletons in small area around p_1. A distance value of p_2' is also replaced ditto with p_1'.

Secondly, d_0, d_1, d_2 and d_3 which are equivalent the distance value of p_0, p_1', p_2' and p_3 are interpolated based on (2), (3).

Finally, the images are reconstructed using connected points and the distance values of them.

The procedure described above, deficiency parts are compensated automatically, and the form of a single neuron is extracted.

IV. Experimental Result

In this section, proposed method is applied to image series of one part of a pre motor neuron. All figures in this section are visualized a three-dimensional using RV-Editor [9].

Fig. 14 is magnified images of a part of Fig. 3. There are many noises in those images. Fig. 15 is as the result of binarization of the images shown in Fig. 14. As shown Fig. 15, there are many deficiency parts appear although noises are cleared away. In this experiment proposed method is applied to the images of Fig. 15. TABLE I shows parameters in this experiment. Those parameters are determined empirically.

Fig. 16 shows a result of selection points from the skeletons which are extracted from the distance transform images of Fig. 15. Fig. 17 shows branching points.

Fig. 18 is the result of connection of points each group with proposed method. In this experiment, the grouping is executed eight times because there are seven branching points as like Fig. 17. Fig. 19 is result of interpolation of the distance values for all group of Fig. 18. Those results show that it is possible to compensate automatically between deficiency parts with smooth curve like the form of a neuron using proposed

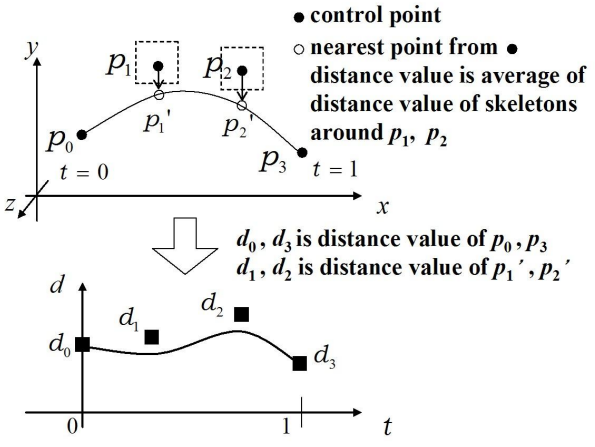


Fig. 13 Interpolating of distance value on Bezier curve

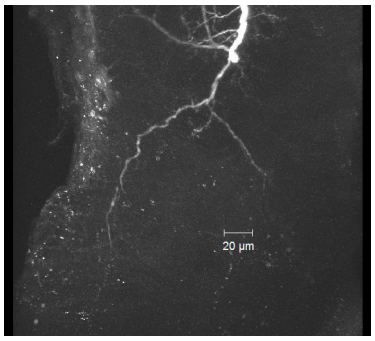


Fig. 14 One part of pre motor neuron

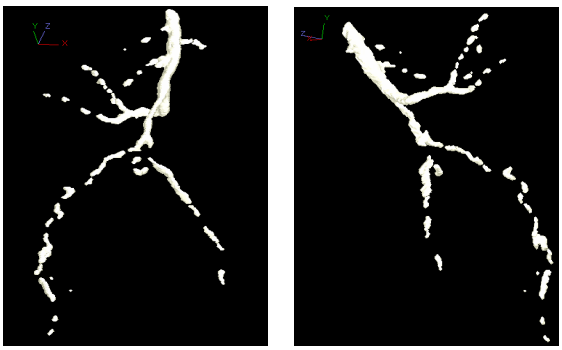


Fig. 15 Binary images of one parts of a pre motor neuron

TABLE I
PARAMETERS IN EXPERIMENTS

Image resolution	1.0μm × 1.0μm × 1.0μm
Image size	x = 237, y = 403, z = 106
Distance of minimum each slate points	10
Number of control points of Bezier curve	5
Step size of Bezier curve	0.0001
Threshold of (1)	0.4
Threshold of d in grouping	$10\sqrt{5}$

method. This enables to extract the form of a neuron, and construct a three-dimensional model. Those processing times are about 10 seconds. It is far speedier than manual operation which several hours taken.

In Fig. 18(e), the angle variation of a curve is widely in large area although it is narrowly in small area. In cases like this, points may be not selected as accurately group, and some groups are not connected at a branching point. Selection of proper points in grouping each branch is very important. In proposed method, angles and distances are employed as indices for grouping. They are weighted, and distances are treated more preferentially. For this reason, the order of selection of points has influence on the result of grouping. Fig. 18(e) shows examples of this problem. In addition, the thicknesses of a neuron vary smoothly in each branch. Therefore, such a grouping mistake extracts improper a form

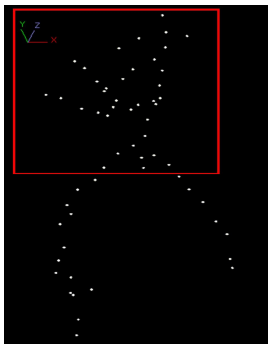


Fig. 16 Selection of points

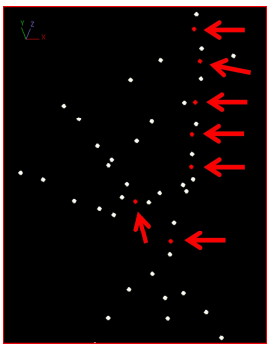
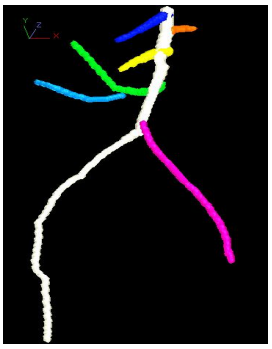


Fig. 17 Branching points



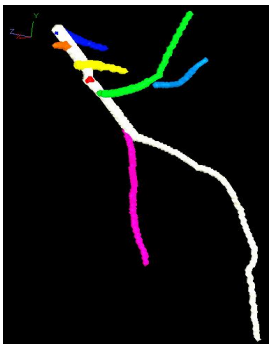
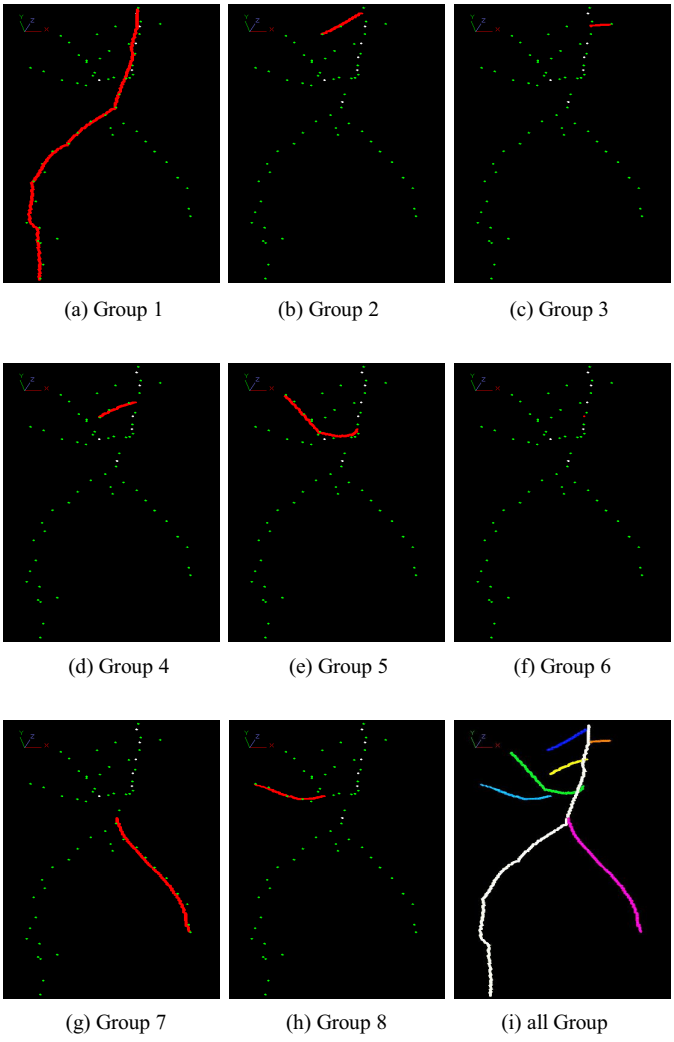


Fig. 19 Interpolation of distance values of Fig. 18



(a) Group 1 (b) Group 2 (c) Group 3

(d) Group 4 (e) Group 5 (f) Group 6

(g) Group 7 (h) Group 8 (i) all Group

Fig. 18 Connection with proposed method

of neuron because the distance values which is thickness of a neuron are interpolated each group in proposed method. This mistake tends to arise near branching points. It is need to consider several combinations of points in small area around a branching point, and select it with validity of those combinations in order to avoid this problem.

V. Conclusion

We presented a method for compensation of deficiency parts through binarization process in order to extract the form of a single neuron. To verify availability of presented method, it was applied to the image series which are captured with CLSM. As the result, it was found that proposed method is effective for compensation of the deficiency parts. Further improvement of our method will achieve an automatic extraction process, and it enables us to reduce the burden of manual processes and save time.

In future works, we will implement index for grouping to connect smoothly with a curve every branch, and devise a method for connection of each curves in the case of branching point has a deficit. In addition it is necessary to estimate quantitatively a connected result.

References

[1] Kanako Nakajima, et al, "Automatic Extraction of the Neurons Form in the Brain of Silkworm Moth", *proceedings of SI2006*, pp.880-881, 2006.

[2] Hiroyuki Ai, Koutaroh Okada, Evan S. Hill, Ryohei Kanzaki, "Spatio-Temporal Activities in the Antennal Lobe Analyzed by an Optical Recording Method in the Male Silkworm Moth Bombyx Mori", *Neuroscience Letters*, 258, pp. 135-138, 1998.

[3] Yoichi Seki, Hitoshi Aonuma, Ryohei Kanzaki, " Pheromone Processing Center in the Protocerebrum of Bombyx mori Revealed by Nitric Oxide-Induced Anti-cGMP Immunocytochemistry", *The journal of comparative neurology*, 480, pp. 340-351, 2005

[4] Takayuki Yamasaki, Teijiro Isokawa, Nobuyuki Matsui, Hidetoshi Ikeno, Ryohei Kanzaki, "Reconstruction and Simulation for Three-Dimensional Morphological Structure of Insect Neurons", *Neurocomputing*, 69, pp. 1043-1047, 2006.

[5] Satoshi Wada, Ryohei Kanzaki, "Neural Control Mechanisms of the Pheromone-Triggered Programmed Behavior in Male Silkmoths Revealed by Double-Labeling of Descending Interneurons and a Motor Neuron", *The journal of comparative neurology*, 484, pp. 168-182, 2005.

[6] K. A. Al-Kofahi, et al, “Rapid Automated Three-Dimensional Tracing of Neurons from Confocal Image Stacks”, *IEEE Trans. Inform. Techno. Biomed.*, Vol. 6, No. 2, pp. 171-187, 2002.

[7] W. He, et al, “Automated Three-Dimensional Tracing of Neurons in Confocal and Brightfield Images”, *Microscopy and Microanalysis*, Vol. 9, pp. 296-310, 2003.

[8] Bernard Katz, “Nerve, Muscle, and Synapse”, *mcgraw-hill series in the new biology*. *McGraw-Hill*, 1966.

[9] `http://www.riken.go.jp/lab-www/V-CAD/katsudo/vcat_team/rveditor/index.html`