Sensor Review

Interpolation of binarized CLSM images for extraction of premotor neuron branch structures in silkworm moth

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Interpolation of binarized CLSM images for extraction of premotor neuron branch structures in silkworm moth

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Abstract

Purpose – The purpose of this paper is to propose an automatic interpolation method for binarized confocal laser scanning microscopy (CLSM) images of a premotor neuron in the silkworm moth.

Design/methodology/approach – Partial deficiencies occur in binary images through the form extraction process because of noises in a CLSM image series. The proposed method selects several points from a binarized image series and connects these points with a Bezier curve based on premotor neuron characteristics in order to interpolate partial deficiencies.

Findings – To verify the availability of the proposed method, a three-dimensional form of a premotor neuron of a silkworm moth was extracted. The results of each branch's relation of connection and of the interpolated neuron thickness show that the proposed method realizes to interpolate partial deficiencies and to extract three-dimensional form of the premotor neuron.

Practical implications – The proposed method contributes to realize efficient premotor extraction process using image-processing techniques. The extracted result by proposed method can be utilized for the form comparison among many data of the premotor neurons quickly. Moreover, it also contributes to provide the parameters of an accurate neuron model for realizing computer simulation of electrical of the neurons.

Originality/value – The proposed method extracts not only a topological form but also a premotor neuron's thickness by interpolating partial deficiencies based on specific characteristics of the neuron. Thickness values of the neuron are an important factor for a simulating accurate electrical response of the neuronal circuit.

Keywords Image processing, Structural analysis, Microscopy

Paper type Research paper

1. Introduction

Insects are flexibly adaptive to environmental changes, but they have far simpler and smaller nervous systems than mammals. Consequently, an insect is one of suitable subjects of the research for analyzing and understanding the neuronal mechanism of environmental adaptation. Therefore, research into structural reconstruction of insect brains has been emphasized to analyze neural networks and mechanisms of information propagation in the brain (Wada and Kanzaki, 2005;

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Sensor Review
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Sensor Review
Volume 29 · Number 2 · 2009 · 137–147

Yamanaka *et al.*, 2005; Ridgel *et al.*, 2007). A typical adaptation behavior is the sex-pheromone search behavior of the male silkworm moth: *Bombyx mori*. This instinctive behavior comprises a well defined series of behaviors: pheromone reception is followed by a surge, a zigzag turn, and a loop. This sequence can be initialized by an additional pheromone reception (Kanzaki *et al.*, 1992; Kramer, 1992). Therefore, trajectories of silkworm moth's locomotion are changeable by pheromone stimuli level such as pheromone concentration.

One of elements generating this pheromone behavior is the premotor neuron and it was reported that only three premotor neurons (G-1) exist in the silkworm moth brain (Wada and Kanzaki, 2005). Therefore, it is thought that such three neurons control pheromone behaviors. However, such behavior generation mechanisms are still not elucidated in detail. Therefore, structural analysis of premotor neurons is important to elucidate such behavior generation mechanisms. Then, the three-dimensional form extraction of the premotor neuron is contributes toward its structural analysis. In this paper, the word form is used to describe shape and topological characteristics.

Generally, it is necessary to capture a cross-sectional image series of the premotor neuron to extract the three-dimensional form of the premotor neuron. This image series is obtained by extracting regions of a fluorescently stained premotor neuron from the image series captured using confocal laser scanning microscopy (CLSM). During the threshold process, which is one of a region extraction process applied to extract fluorescently stained regions, some partial deficiencies occur, and they become critical problem for structural analysis of the neuron. Therefore, it is required to interpolate partial deficiencies in the image series. This interpolation process curried out manually. For that reason, it is very hard operation and it is difficult to process vast quantities of the images. Furthermore, the interpolation accuracy depends on personal empirical capability.

In this paper, we propose a method for automatical partial deficiencies interpolation in the binarized CLSM image series. Proposed method realizes the interpolation by selecting several points from images and connecting these points with smooth curves to extract the form of the premotor neuron.

2. Form extraction of a single neuron

2.1 Capture of the cross-sectional image series

A cross-sectional image series of a fluorescently stained neuron is utilized to extract a three-dimensional model of the neuron form. For this study, the cross-sectional image series are reconstructed as three-dimensional data: voxel data. The image series is captured through the following steps (Seki *et al.*, 2005):

- Impale an intended single neuron on a glass microelectrode filled with a fluorescent dye.
- Apply a 1-10 nA electrical current to a glass electrode for injection of the dye into the neuron.
- Fix the brain in formaldehyde, dehydrate it using an ethanol series, and clarify it using methyl salicylate to obtain high-S/N samples.
- Capture the cross-sectional image series of a single neuron using CLSM.

In the steps described above, because the central axis of images is not out of alignment, it is possible to capture high-quality images. Figure 1 shows the silkworm moth brain and Figure 2 also shows the appearance of injection of a fluorescent dye into a single neuron in the silkworm moth brain. Figure 3 illustrates a schematic diagram of capturing an image series with CLSM; Figure 4 indicates the example of a cross-sectional image series obtained using CLSM. Figure 5 presents a projection image of the cross-sectional image series.

2.2 Issue in form extraction using the cross-sectional image series captured using CLSM

Approximately, 10^3 data of the CLSM image series exist in our database. Extraction of the fluorescently stained region from such image series enables a three-dimensional form extraction of a single neuron.

In some previous works, automatic form extraction methods were examined. Al-Kofahi *et al.* (2002) proposed

Figure 1 Silkworm moth brain

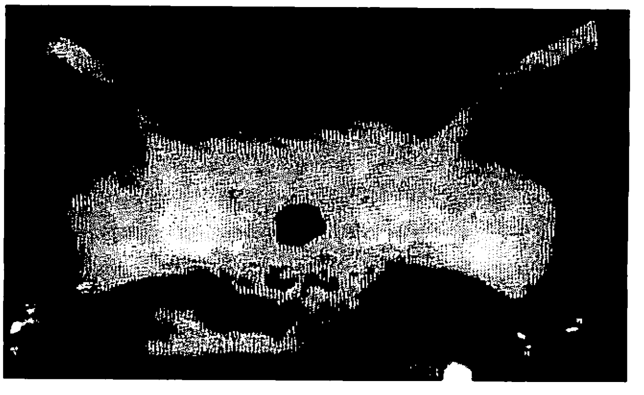


Figure 2 Injection of a fluorescent dye into a single neuron

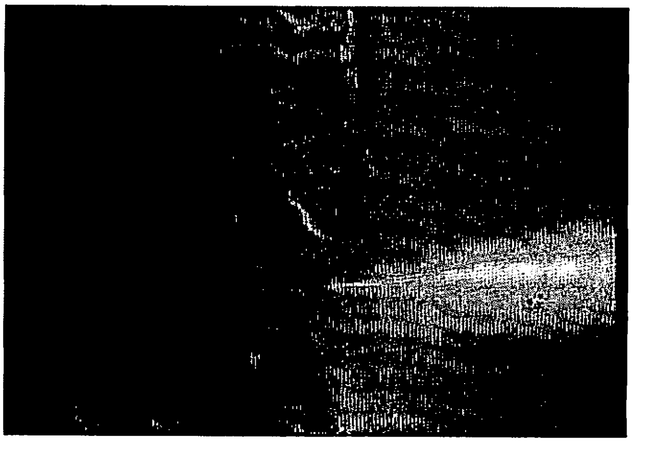
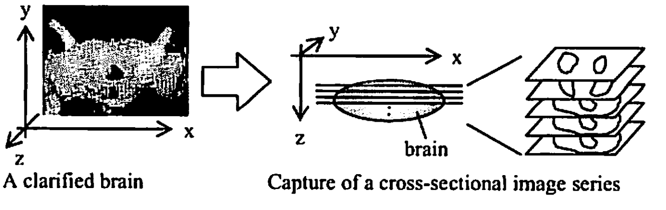


Figure 3 Capture of the cross-sectional image series by CLSM



Sensor Review
Volume 29 · Number 2 · 2009 · 137–147

Figure 4 Example of a cross-sectional image series with CLSM

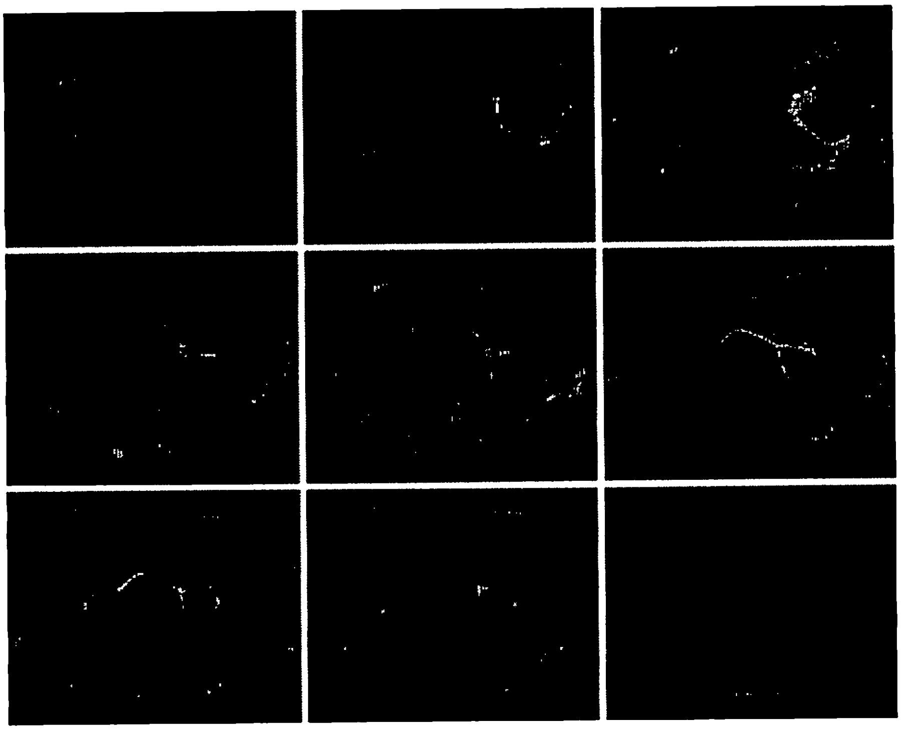


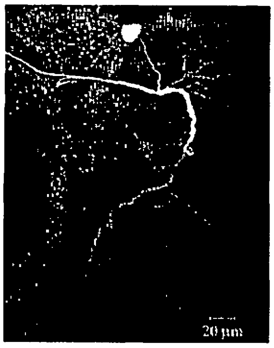
Figure 5 Projection image of the cross-sectional image series with CLSM

the method using a cylinder model of the neuronal structures, He *et al.* (2003) use distance and path tortuosity of a neuron's branch, Yamasaki *et al.* (2006) also use single-seed distance transformation, and (Urata *et al.*, 2007) discussed the threshold, the labeling, and the erosion process. Notwithstanding, these methods are applicable only to images without any noise and (Yamasaki *et al.*, 2006; Urata *et al.*, 2007) are useful in extraction of a thick main dendrite. Moreover, He *et al.* (2003), Yamasaki *et al.* (2006) require manual procedures in some processes. Al-Kofahi *et al.* (2002) and He *et al.* (2003) are used merely for extraction of a topological characteristic of a neuron: neuron thickness cannot be extracted. Electrical responses of a neuron are often analyzed with computer simulations based on a reconstructed form of a neuron (Yamasaki *et al.*, 2006). The expression site and transfer rate of the action potential depend on the neuron thickness (Katz, 1966). Therefore, neuron thickness is important information to simulate for neuron structure analysis.

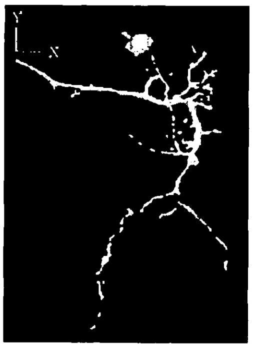
In addition, there are many methods exist for segmentation of vascular form in a Magnetic Resonance Angiography (MRA) cross-sectional image series. Gooya *et al.* (2007) proposed a method using a flux maximizing scheme, Sekiguchi *et al.* (2004) discussed branch-based region-growing method, and (Yan and Kassim, 2006) also proposed the common physical phenomenon of capillary action in thin vessels. However, MRA images include only few noises.

The captured cross-sectional image series with CLSM has a difference of brightness resulting from dyeing unevenness, optical noise, and autogenic fluorescence. Especially, it is difficult to eliminate noises of autogenic fluorescence from the captured image series because there are little differences between noises and weak signal parts of a neuron and noises are spread throughout in images. For these reasons, it is difficult to extract only the fluorescently stained region with previous methods. Figure 6 shows typical partial deficiencies. In our current research, partial deficiencies are defined as disconnected parts after threshold processes: noise reduction processes, for extraction neuron's form although they are actually connected. In most case of the vascular form extraction, the vessel has thick branches rather than general neurons' one and usually is enough to extract the form of thick vessels for evaluating the vascular structure. It does not become critical problem not to consider the partial deficiencies in thin vessels. However, partial deficiencies make it impossible to analyze structure of a neuron because the neuron has many partial deficiencies in some locations. Therefore, it is often the case that we must retouch the partial deficiencies manually using photograph-retouching software. In case of all most data stored in our data base, partial

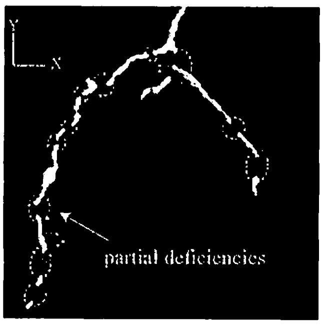
Figure 6 Example of partial deficiencies through the threshold process (a projection image)



(a) The CLSM image series



(b) The binarized CLSM image series



(c) Partial deficiencies (magnified view of Figure 6(b))

deficiencies occur after the threshold process. Then automation of the interpolation process is required. In addition, it is necessary to interpolate partial deficiencies based on specific characteristics of a neuron to analyze its structure. Therefore, we must consider the thickness of a neuron for automatic interpolation. Interpolating partial deficiencies, the neuron form can be extracted.

The subject of this study is to restore the premotor neuron form from the image series with partial deficiencies. The method proposed herein is designed to extract it.

3. Proposed method

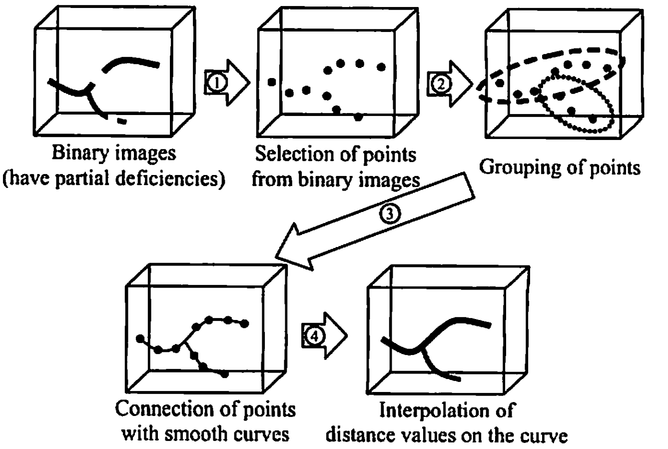
3.1 Interpolation of partial deficiencies by selecting and connecting of points

The binarized CLSM image series include no noise after threshold processes, as premises for the proposed method. It is difficult to distinguish a background region from a partial deficiency region in the binarized cross-sectional image series through the threshold process because this image series includes many partial deficiencies. Hypothesizing that partial deficiency regions are connected for interpolation of partial deficiencies is treating partial deficiency regions and extracted regions by the threshold process equally. Consequently, we make extracted regions by the threshold process be full of partial deficiencies. Herewith we can think simply this interpolation problem.

By these reasons, we consider the image series not as neighboring points but as sets of distant points, and connect these points with smooth curves. Figure 7 presents a schematic diagram of the proposed method.

First, several points are selected from regions that are not background. These images are expressed only with points.

Figure 7 Schematic diagram showing the proposed method



This yields similar sets of points, irrespective of whether they are with or without partial deficiencies and irrespective of their location.

Second, these points are grouped for every branch and are connected with a smooth curve for every group. From these operations, partial deficiencies are interpolated. The neuron thickness is similarly interpolated on curves.

Finally, the neuron form is extracted using connected curves and the neuron thickness. Details of each procedure are described in the following sections.

3.2 Details of the proposed method

3.2.1 Selection of points from binary images

We describe the process for selection of points using a two-dimensional model for ease of explanation although this process is actually performed with a three-dimensional one.

First, a binarized image series is transformed with a distance transform image. It is a process to transform a pixel value of a binarized image into a Euclidean shortest distance from background pixels. Figure 8 shows an example of a distance transform image.

Second, skeletons are extracted from the distance transform image. A skeleton is a central pixel of a circle when covering an image with a circle of necessary minimum. It is possible to reconstruct foreground regions perfectly using skeletons and their distance value (Saito and Toriwaki, 1993). Figure 9 depicts an example of extraction of skeletons from a distance transform image; Figure 10 shows results of skeleton extraction from Figure 8. We consider distance values of skeletons as the neuron thickness because it denotes the diameter of an inscribed circle. For three-dimensional processing, a skeleton is a central pixel of an inscribed sphere.

Finally, several points are selected from skeletons in regular intervals based on a maximum size of partial deficiencies. Skeletons are not always aligned on the centerline of the neuron because the neuron surface is convexo-concave. Consequently, points are selected from skeletons that are proximal to the centerline, which is obtained from the thinning process based on the distance value of distance transform images (Saito *et al.*, 1996). This yields a similar set of points irrespective of whether they are with or without partial deficiencies, and irrespective of their location. In addition, our method does not consider smaller branches than the sampling step.

Figure 8 Example of a distance transform image (two-dimensional)

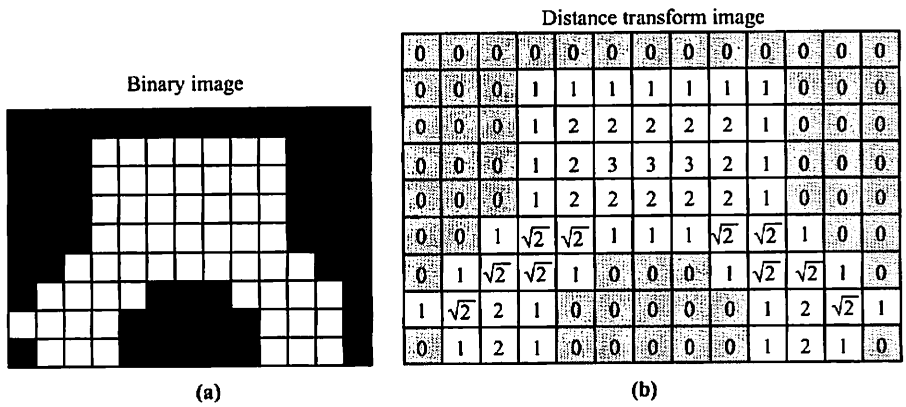


Figure 9 Extraction of skeletons

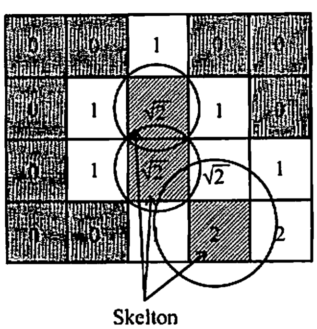
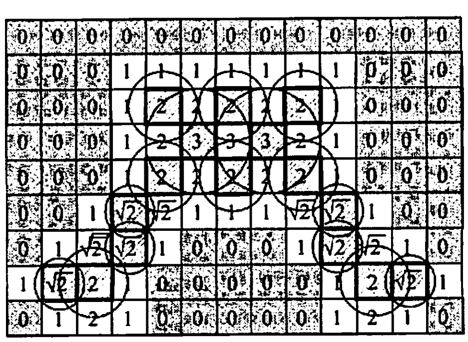


Figure 10 Extraction of skeletons from Figure 8

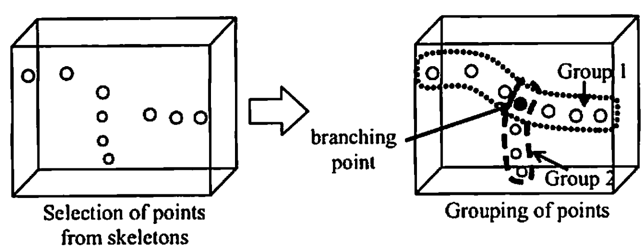


3.2.2 *Grouping of points every branch*

Figure 6 shows that the premotor neuron has many branches; thin branches diverge from one thick branch. In addition, the branch curvature is smooth. The thickness varies among branches. Therefore, it is appropriate that points be grouped at every branch and be interpolated with a smooth curve for every group.

Selected points from skeletons are grouped for every branch, as shown in Figure 11. Points with the same group label appear closely; the angle variation formed by four points and the thickness variation is small. Consequently, one group is a set of combinations for which the angle variation is smallest and the thickness variation is smallest in a local area. In addition, the number of points in a combination is used as an evaluation index of grouping. If the distance between two points is greater than d, these points do not belong in the same group because the distance of two points that belong in same group is not so long.

Figure 11 Grouping of points at every branch



3.2.3 *Connection of points with smooth curves*

Points are not always aligned on the centerline because these points are selected from skeletons. For this reason, it is best to avoid producing a curve passing through all points. A Bezier curve passes only through a start point and an end point, and connects points with control of other points. Therefore, we use a Bezier curve as a connection method. The Bezier curve is drawn based on the following equations:

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

$$B_i^n(t) = \binom{n}{i} t^i (1-t)^{n-i}. \tag{2}$$

Therein, n is the number of control points in the Bezier curve, t is the number of the step size of the Bezier curve. In addition, $p_i = (x_i, y_i, z_i)^T$ indicates the position vector of points which are selected from skeletons; the value of x, y, and z are each obtained with equation (1).

This grouping and connection of points are processed sequentially every branch. The first grouping starts at an end point of a thick branch, and points that belong to this group are connected. After the first grouping, grouping starts at a branching point. This branching point is replaced with a skeleton that is proximal to a branching point, which is extracted through the thinning process (Saito *et al.*, 1996). It is thought that this is because branching points are deficient if there are some points that are not connected after grouping and connections that are started at an end point or a branching point are all finished. In that case, it is necessary to give branching points. This branching point is replaced with a point that is the nearest point from among the remainder of points on a curve, and grouping is started at this branching point.

Finally, after grouping and connection at all points are finished, interpolation between curves is performed by connecting the branching point and the nearest curve.

3.2.4 Interpolating of distance value on the curve

Partial deficiencies can be interpolated using the procedures described above. However, these procedures only realize connect points with a curve for every branch; it is nothing more than extraction of topological characteristics of the neuron. Then, in this section, determining the distance value of points which are on curves for every branch, the neuron thickness is interpolated and it is able to extract the form of the neuron.

Distance values of control points of the Bezier curve are defined certainly because these points are selected from skeletons. On the other hand, distance values of other points on curves are not necessarily defined. Distance values on curves should be interpolated with distance values of control points of Bezier curve and their surrounding skeletons, as shown Figure 12. In Figure 12, p_1 is shown as a Bezier curve start point and p_4 is an end point; d_1 is the distance value of p_1.

First, the distance value of p_2', which is the nearest point from p_2 on the curve, is decided. The distance value of p_2' is replaced with an average value of distance values of skeletons in a small area around p_2. The distance value of p_3' is replaced similarly with p_2'.

Second, distance values between d_1, d_2, d_3, and d_4, which are equivalent the distance value of p_1, p_2', p_3', and p_4, are interpolated based on equations (1) and (2). Actually, d corresponds to p in equations (1) and (2).

Finally, the image series is reconstructed using connected points and their distance values.

With the procedures described above, partial deficiencies of the binarized image series are interpolated automatically; the three-dimensional form of a premotor neuron is extracted automatically with just given a binarized CLSM image series.

4. Experimental results

The proposed method was applied to the image series of one part of a premotor neuron to verify the availability of the method. We curried out experiments using three data sets which are shown on Figure 13. Figure 13(a) is the binarized image series against Figure 6(a); many partial deficiencies are apparent. Table I shows parameters used in this experiment.

Figure 12 Interpolating distance values on a curve

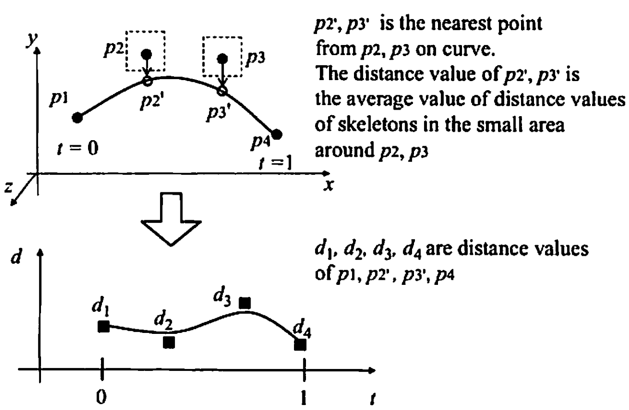
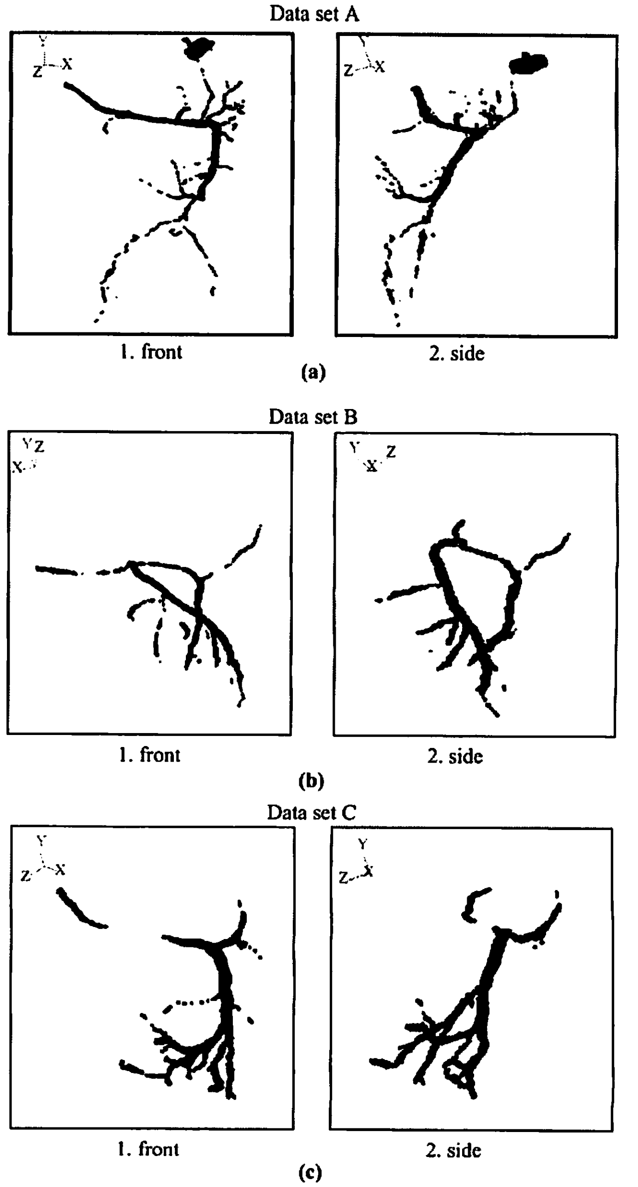


Figure 13 The binarized image series of one part of the premotor neuron



These parameters are determined empirically. In these experiments, points are selected in regular intervals based on the second largest size of partial deficiencies because the largest one is far larger than other partial deficiencies. All figures in this section are visualized three-dimensionally using V-Cat which is software for three-dimensional visualization (RIKEN, 2004).

Figure 14 depicts interpolation results of Figure 13 and Table II presents connection results of each branch. These results show the proposed method realize to interpolate partial deficiencies and extract a form of a premotor neuron.

We examine extraction results for example in the result of Figure 13(a). Figure 15 displays a result of selection of points from skeletons that are extracted from the distance transform image series of Figure 13(a). Figure 16 depicts a magnification of Figure 14: it shows branching points. Figure 17 shows a connection result of points that belong to

Table I Parameters used in experiments

Image resolution		**1.0 × 1.0 × 1.0 μm**
Image size	Data set A	$x = 237$, $y = 403$, $z = 235$
	Data set B	$x = 260$, $y = 268$, $z = 157$
	Data set C	$x = 283$, $y = 305$, $z = 170$
Distance of each point (DEP)	Data set A	10
	Data set B	6
	Data set C	6
Size of local area in grouping		$\mathrm{DEP} \times \sqrt{5}$
Number of control points of Bezier curve		3
Step size of Bezier curve		0.0001
Threshold of d in grouping processes	Data set A	$\mathrm{DEP} \times \sqrt{\mathrm{DEP}/2}$
	Data set B	$\mathrm{DEP} \times \sqrt{\mathrm{DEP}}$
	Data set C	$\mathrm{DEP} \times \sqrt{\mathrm{DEP}}$

Figure 14 Extraction results of a premotor neuron

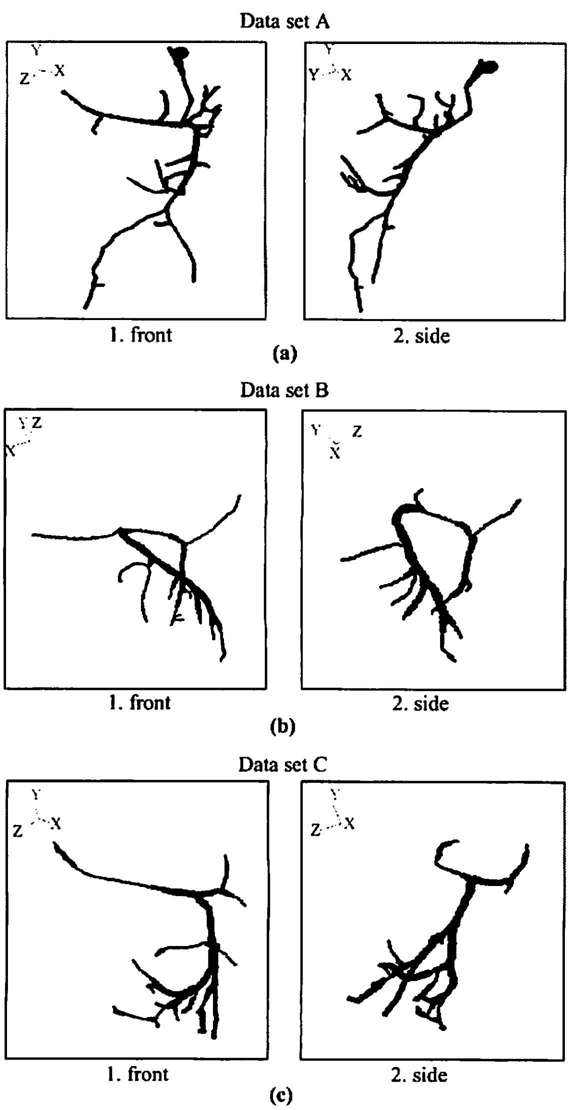


Table II The number of branches

	Accurate all branches	Connected all branches	Correctly connected branches
Data set A	22	23	19
Data set B	10	11	10
Data set C	13	13	13

Figure 15 Selection of points (data set A)

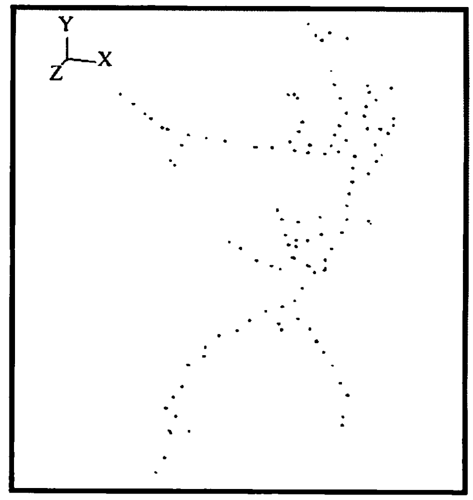


Figure 16 Branching points (magnified view of Figure 14)

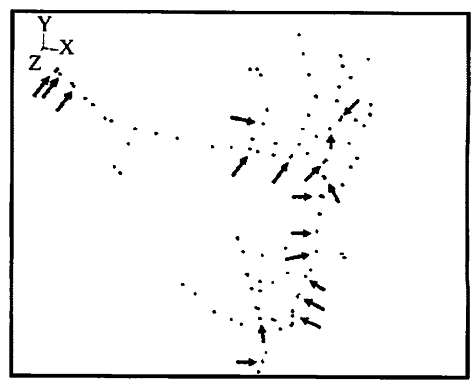
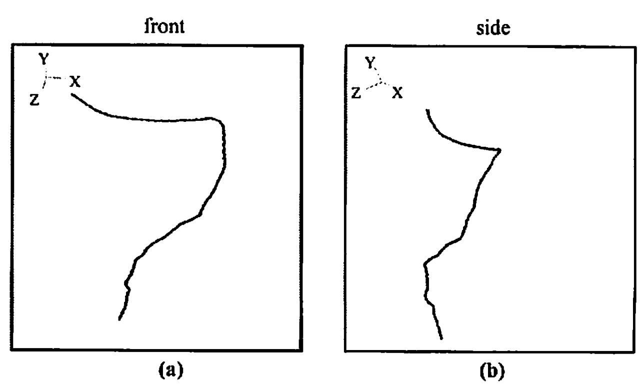


Figure 17 Connected result of a thick branch (data set A)



Sensor Review
Volume 29 · Number 2 · 2009 · 137–147

a thick branch in Figure 15. It shows that the proposed method can select and connect only points belonging to a thick branch from Figure 15. Figure 18 portrays the result of grouping and connecting when a start point of grouping is an end point and a branching point. It is possible to group and connect points at every branch using the proposed method. Figure 19 shows that some points are remaining without grouping and connection because branching points are deficient. Then, Figure 20 depicts a result of grouping and connection against the remaining points, and connection of each branch. It shows that all points are connected using the proposed method. In addition, Figure 14(a) shows the result of interpolation of a premotor neuron's thickness.

Figure 18 Connected result showing all branches that have branching points (data set A)

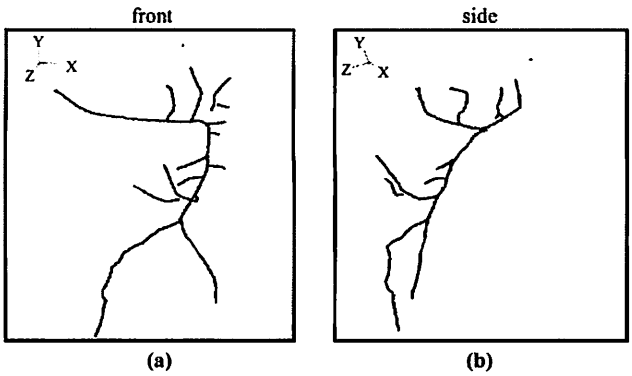


Figure 19 Case in which branching points are deficient (data set A)

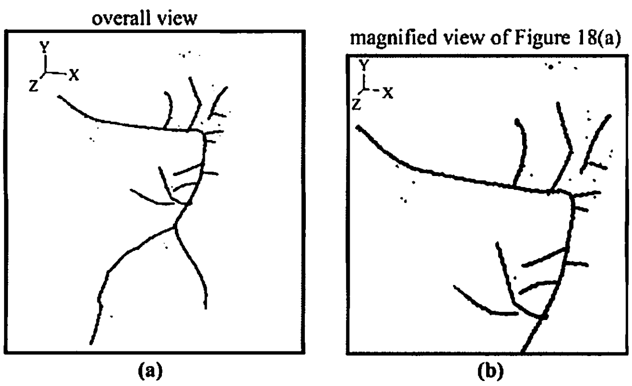
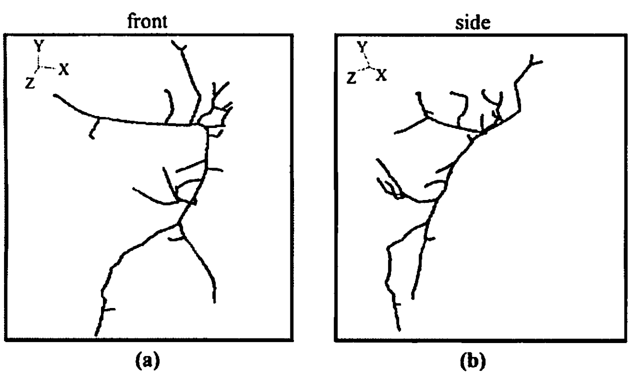


Figure 20 Connected result of all points (data set A)



These results demonstrate that it is possible to interpolate partial deficiencies and thickness of the premotor neuron of binarized CLSM image series automatically, and to extract a three-dimensional form of the premotor neuron using the proposed method.

These processing times are about 40 s. It is far speedier than manual operations, which require several hours or days.

Comparison of Figure 13 with Figure 14(a) shows that the interpolated result using the proposed method is a proper result with a visual estimate. This result is compared with an image that was interpolated manually to evaluate the connection of relation of a neuron in results obtained using the proposed method. Figure 21 shows results that are interpolated manually, with an applied the thinning process. This manual process can retouch partial deficiencies by superimposing a binary image on an original image; it is done separately from the proposed method. Table III presents the number of all branches and the number of branches that deliquesce from a thick branch in each result. It shows that the numberof branches is different. Then relations of connection of branches in these results are compared using a tree structure expression (Kobayashi *et al.*, 2007) to evaluate the connection of the relation of branches.

In these tree structure images, nodes are branching points and edge points.

Figure 22 shows images that are represented by the tree structure for each interpolated result. Regions in Figure 22(a) and (b) correspond with regions in the structure of interpolated images in Figures 23 and 24. Figures 22-24 show that the interpolated results obtained using the proposed method are equal to the results obtained by manual interpolation in connection relations, except for certain regions.

5. Discussion

In Figures 22-24, branches which are indicated by the arrows are different results of connection of relation.

This shows the regions for which the connection of relations of branches is wrong that have deficient on a branching point.

Figure 21 Result of manual thinning process in the interpolated result (data set A)

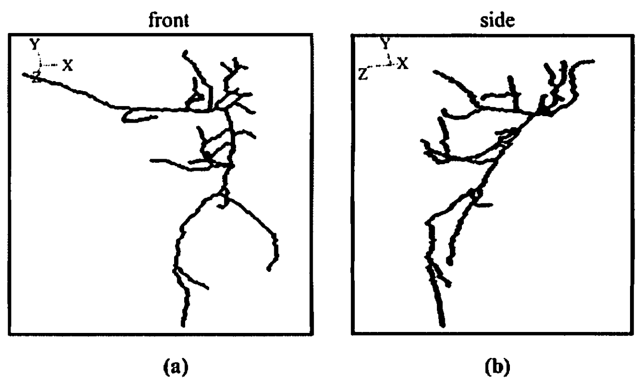
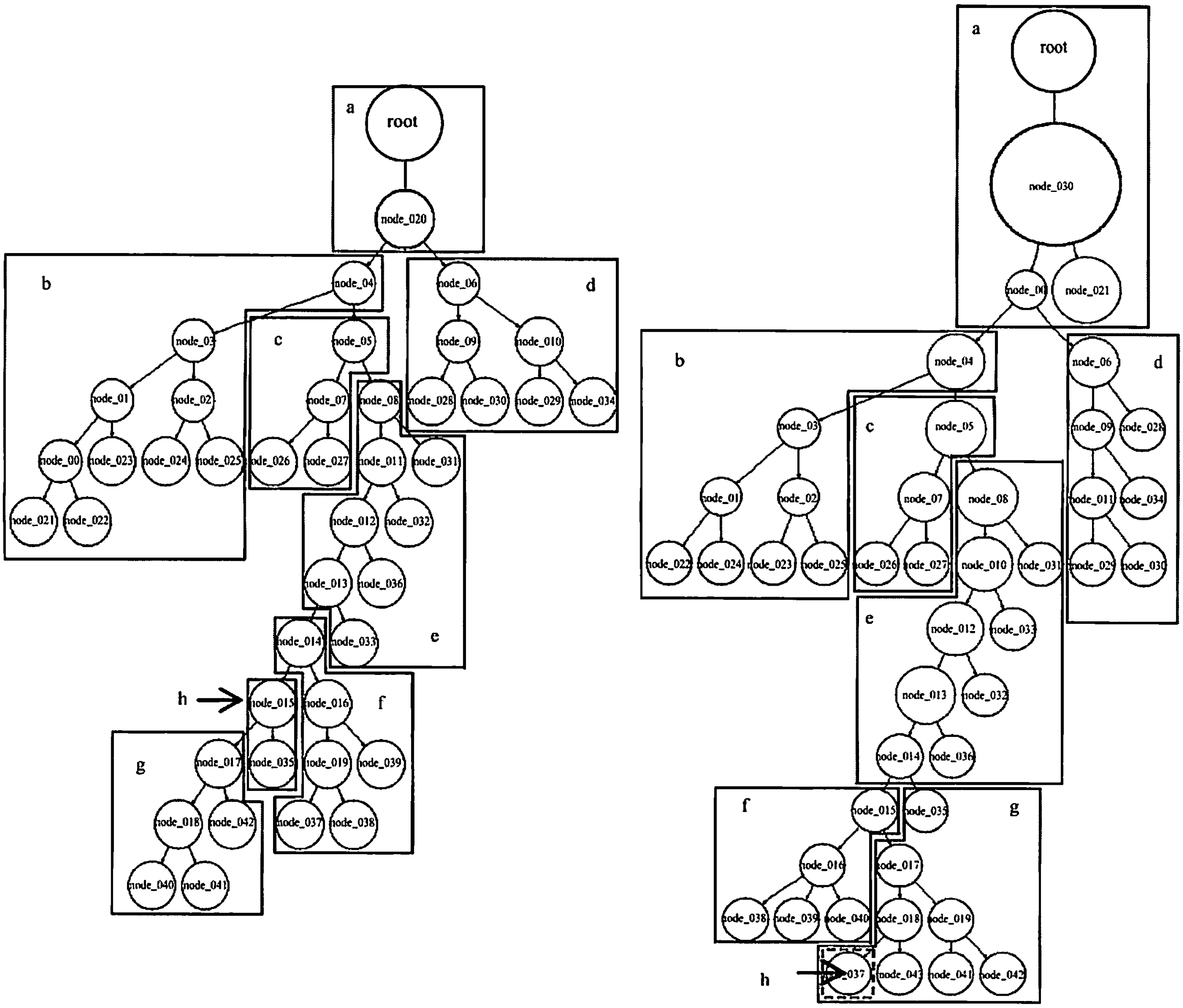


Table III Connection relation result

	Manual	Proposed method
Number of branches	22	23
Number of branches which deliquesce from the thick branch	13	14

Sensor Review
Volume 29 · Number 2 · 2009 · 137–147

Figure 22 Comparison of interpolation result with the tree structure (data set A)



(a) Manual result

(b) Proposed method

In addition, we got the same results in experiments on two of other image series; interpolation by proposed method is done when branching points are not deficient. This problem is difficult to avoid because the proposed method connects each branch with branching points. It is thought that this problem is avoidable by giving branching points beforehand, or demanding branching points by collating with an original image or image that is obtained using a low threshold process.

Comparison of Figure 13 with Figure 14 shows that the proposed method is effective to interpolate the neuron thickness with a visual estimate. However, interpolation results of it in around the cell body and part of the thick branch are insufficient. It is thought this is because the premotor neuron's form is not a columnar form and many skeletons exist around of the centerline; the neuron thickness cannot be expressive only distance values of skeletons that are proximal to the centerline. Then a method using cross-sectional area of a neuron should be effective to avoid this problem. Additionally, it is necessary to estimate the neuron thickness quantitatively.

6. Conclusion

We presented a method for interpolation of partial deficiencies of a binarized CLSM image series through an extraction process to extract the three-dimensional form of the premotor neuron. The proposed method selects several points in every uniform gap from a binarized image series and connects these points with smooth curves based on premotor neuron characteristics: it has numerous branches and thin branches diverging from one thick branch; the branch curvature is smooth and its thickness varies among branches. It was applied to the binarized image series of the premotor neuron to verify the availability of the presented method. The results show that the proposed method realizes to interpolate partial deficiencies automatically and to extract

Figure 23 Structure in local region done manually (data set A)

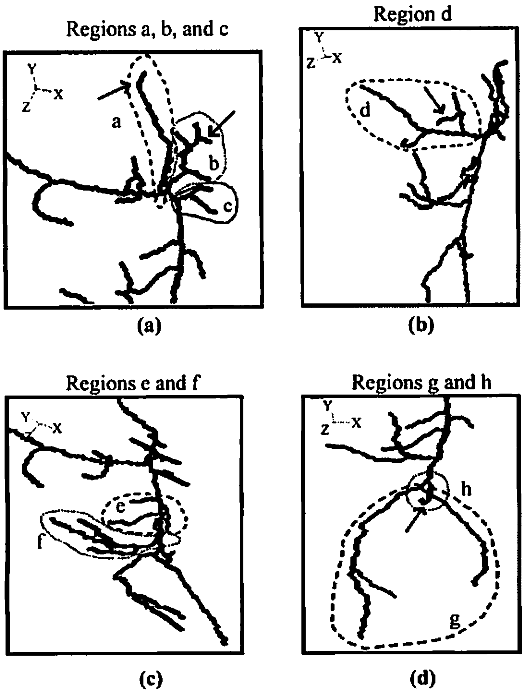
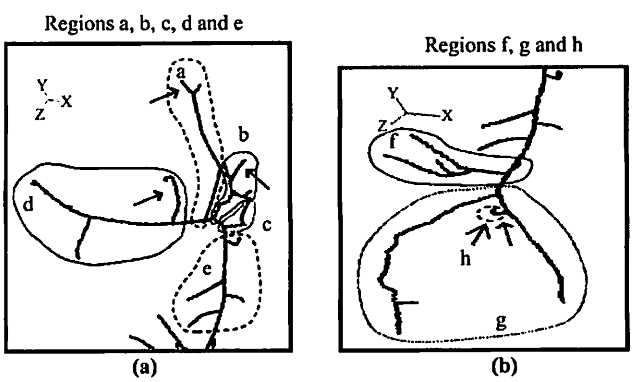


Figure 24 Structure in local region using the proposed method (data set A)



the three-dimensional form of the premotor neuron. It is possible to ease the burden of manual processes and reduce time during a form extraction processing. Connection relations of the result which interpolated using the proposed method are evaluated through comparison with the manual interpolated result with the tree structure. Results show that both results are equal in connection relations if branching points are not deficient.

For improving of our method, it is thought that evaluating relation of connection of an interpolated result and giving appropriate feedback is effective. Additionally, in our method, devising an evaluation index of grouping enables application to other kinds of neurons.

Future works should consider methods for replacing branching points when these points are deficient, and for interpolation of the neuron thickness. In addition, the proposed method is applicable to a lot of other image series to analyze differences of form construction in three premotor neurons.

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