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Interpolation of the Cross-sectional Area of a Premotor Neuron in a Silkworm Moth Brain using the Ellipse Model

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Abstract—This paper presents a proposal of a method for interpolation of cross-sectional area of a premotor neuron in a silkworm moth brain for form reconstruction of the neuron. We have proposed a method for the automatic interpolation of partial deficiencies which occur in extraction processes of the form of a neuron because of noise in CLSM images. However, because this method presupposes approximation of a neuron form as a cylinder model, interpolation of a cross-sectional area of a neuron is insufficient. The cross-sectional area of a neuron yields important information for structure elucidation of the neuron because the expression site and transfer rate of the action potential depend on the cross-sectional area of a neuron. The cross-sectional area is interpolated using the proposed method, which approximates the cross-sectional area of a neuron using the ellipse model. The proposed method applies the task of interpolation of the cross-sectional area of the artificial images to verify the availability of the method.

I. INTRODUCTION

Adaptative ability to an environment is a necessity of all animals to survive. This capability is also necessary for autonomous systems and Intelligent system, especially for robotic systems. Therefore, understanding the mechanisms underlying the environmental adaptation of animals will be a great help for progress in robotics.

Insects can be flexibly adaptive to changes in the environment, although they have far simpler and smaller nervous systems than mammals do. Consequently, insects are suitable models for analyzing and understanding 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. A simple adaptation behavior is the sex-pheromone search behavior of the male silkworm: *Bombyxmori*. This instinctive behavior comprises a well defined series of behaviors: pheromone reception is followed by a surge, a zigzag turn, and a loop [1], [2]. This sequence can be reset by

an additional pheromone reception. Therefore, trajectories of locomotion are shaped by pheromone circumstances such as pheromone concentration.

One neuron controlling this behavior is the premotor neuron. The premotor neurons (G1) in the brain are just three [3]. For that reason, structural analysis of the premotor neuron is important to elucidate behavior mechanisms. Form reconstruction of the premotor neuron is essential for its structural analysis. In this paper, form means shape and topological characteristics. It is necessary to capture the cross-sectional image series of a premotor neuron to reconstruct the form of the premotor neuron. This image series is obtained by extracting a fluorescently stained region from the image series captured using CLSM. During the threshold process applied to extract them, some partial deficiencies occur. Because those deficiencies constitute a fatal problem for structural analyses, it is necessary to compensate these partial deficiencies. Currently, the process is performed manually. For that reason, it is a laborious task, and it is difficult to process vast quantities of data. In addition, the compensation result depends on personal ability.

We have proposed a method for the automatic interpolation of partial deficiencies in binary images with the selection of discrete points and their connections [4]. Use of this method enables interpolation of partial deficiencies and representation of the form of neuron with smooth curves. This method presupposes that it is possible to approximate a neuron form as a cylinder model. However, it is often the case that surface of a neuron is patterned indented. In this case, interpolation of the cross-sectional area of a neuron is insufficient. We propose a method for interpolation of the cross-sectional area using an ellipse model.

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 necessary to construct a three-dimensional model of a single neuron. This image series is captured through the following steps [2].

1) Impale an intended single neuron with a glass microelectrode filled with a fluorescent dye.

2) Apply a 1–10 nA electrical current to a glass electrode for injection of the dye into the neuron.

3) Fix the brain in formaldehyde, dehydrate it with an ethanol series, and clarify it using methyl salicylate to obtain high-S/N samples.

4) Capture the cross-sectional image series of a single neuron using confocal laser scanning microscopy (CLSM).

In those steps, the central axis is not out of alignment. Therefore, we can capture high-quality images. Fig. 1 depicts the silkworm moth brain. Fig. 2 shows the appearance of injection of a single neuron in the silkworm brain. Fig. 3 is a schematic diagram of capture of a cross-sectional image series with CLSM. Fig. 4 is a projection image of a premotor neuron. For this study, these image series are reconstructed as three-dimensional data.

B. Extraction of the form of a single neuron

Extraction of the fluorescently stained region enables construction of a three-dimensional model of a single neuron. However, the CLSM image series has a difference of lightness resulting from dyeing unevenness, optical noise, and autogenic fluorescence. For these reasons, it is difficult to extract only the fluorescently stained region; partial deficiencies occur through extraction processes like those portrayed in Fig. 5. Although there are about 10^3 data in our database of CLSM images, partial deficiencies through the extraction process occur in most of them. Therefore, it is often the case that we must retouch the partial deficiencies manually using photo retouching software.

Previous works include some studies that have examined automatic extraction of the form of a neuron. For example, one method uses a cylinder model of the neuronal structures [5]; another uses distance and path tortuosity [6] and single-seed distance transform (SSDT) [7]. Notwithstanding, these methods are applicable only to images which include no noise; moreover, they require manual procedure in some processes. They are used merely for extraction of a topological characteristic of neuron: the neuron thickness cannot be extracted. The expression site and transfer rate of action potential depend on the neuron thickness [8]. Therefore, the neuron thickness is important information to analyze neuron structure. For these reasons, we propose a method that enables automatic compensation of the partial deficiencies and extract the form of a neuron with involvement a thickness

Fig. 4. The projection image of a premotor neuron

(a) CLSM images   (b) Binary images
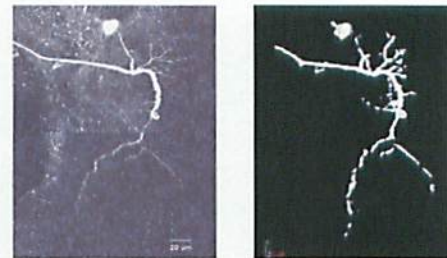
Fig. 5. An example of partial deficiencies through the threshold extraction process (projection images)

of a neuron.

C. Compensation of partial deficiencies with Selection and Connection of discrete points

It is difficult to define edge points of the neuron because the binary cross-sectional image series through the threshold process has many partial deficiencies and many edge points. Therefore, we consider the binary image series not as contiguous voxels, but as sets of discrete points; those points are connected with a smooth curve [4]. These procedures include the following steps; Fig. 6 presents a schematic diagram of each step. In this method, the binary image series has no noise through the threshold process.

1) *Extraction of discrete points*

The binary images are transformed to the Euclidean distance images and skeletons are extracted from these Euclidean distance images. The Euclidean distance images

Fig. 1. The brain of silkworm moth   Fig. 2. Injection of a single neuron

A clarified brain   Capture a cross-sectional image series

Fig. 3. The cross-sectional image series with CLSM

Binary images (have deficiency parts)   Selection of points from binary images   Grouping of points

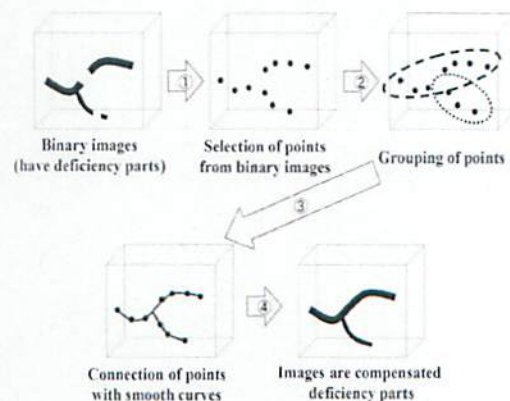Connection of points with smooth curves   Images are compensated deficiency parts

Fig. 6. Schematic diagram of method for compensation of partial deficiencies

are transformed to a voxel value of binary images into the shortest Euclidean distance from background voxels. The skeletons are a set of central voxels of spheres when covering foreground regions with a minimal number of spheres. These spheres are inscribed spheres and the sphere diameter is a distance value of the central voxel. For that reason, the distance value is equivalent to the neuron thickness. It is possible to reconstruct foreground regions using all skeletons and their distance values.

Finally, several points are selected from skeletons discretely. Those points are selected in every uniform gap based on a maximum size of partial deficiencies. This obtains similar sets of points, irrespective of whether they are with or without partial deficiencies and irrespective of their location.

2) Grouping of discrete points at every branch

The premotor neuron has branches; their thickness varies in every branch. In addition, the branch curvature is smooth. For these reasons, after discrete points are grouped in every branch, discrete points and neuron thicknesses are interpolated with smooth curves in every group. This grouping is based on the distance of two points, the angle between a certain point and the neighboring one, and the variation of thickness.

3) Connection of discrete points with a smooth curve for every branch

Points which are grouped for every branch are connected with a Bezier Curve in every group. Grouping and interpolation are processed sequentially. In the first grouping, the start point of grouping is the end point. In the second and later groupings, the start point is a branching point which is extracted through thinning process. This branching point is replaced with a skeleton, which is proximal to a branching point.

After grouping is completed, interpolation between each curve is performed by connecting branching points and the nearest curve.

4) Interpolation of the neuron thickness and reconstruction of the neuron form

Although the distance value of control points of Bezier curve in connection of deficiency points is defined, the distance value of other points on the Bezier curve is not necessarily defined. These distance values are interpolated with the distance value of the control point and the Bezier curve.

Finally, images are reconstructed using spheres; central voxels are interpolated points and the sphere diameter is their respective distance values.

D. The form of a premotor neuron

A neuron thickness is determined with the distance value of points on the assumption that a neuron form is approximated using a cylinder model in our previous method. However, there are many skeletons around a center line and surface of a neuron beacause surface of a neuron is concavo-convex, as shown in Fig. 7. For this reason, interpolation of the cross-sectional area of a neuron is insufficient with
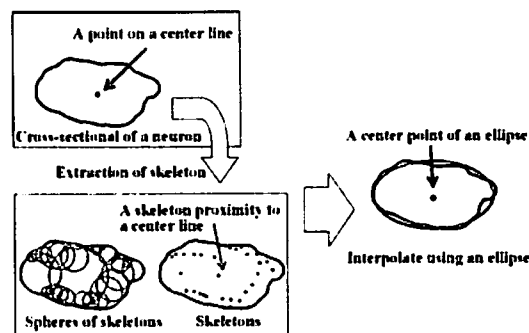

Fig. 7. Example of a cross-sectional surface of a neuron

our previous method; using only a distance value of a skeleton which is proximity to a center line. Then the cross-sectional area is interpolated using the proposed method, which approximates the cross-sectional area of a neuron using an ellipse model.

III. INTERPOLATION OF THE CROSS-SECTIONAL AREA OF A PREMOTER NEURON USING AN ELLIPSE MODEL

The cross-sectional area of a neuron is not any less necessary for further study than the form for structural analysis of a premotor neuron and simulation of its action potential. Approximation of the cross-sectional area of a neuron using not an inscribed circle model, but an ellipse model is appropriate because of characteristics of a premotor neuron: the surface is hubbly. Parameters which are necessary for an ellipse model are obtained with binary images and the center line of which is extracted through a thinning process based on a distance value from background voxels [9].

First, the binary images are transformed into Euclidean distance images and the center line is obtained. In addition, discrete points are selected from the center line.

Secondly, tangent vectors of the center line are determined. When the plane perpendicular to this tangent vector is the cross-sectional surface of a neuron (Fig. 8), the area of this cross-sectional surface is obtained with number of points on this cross-sectional surface. Branching points have three tangent vector. Then, in branching points, a tangent vector obtains smallest cross-sectional area in three. In proposed method, this cross-sectional surface is approximated by an ellipse model. A central point of the ellipse is replaced a median point of points on the plane of the cross-sectional surface. In addition, the long axis and the short axis are obtained by eigenvalues and eigenvectors of a variance-covariance of coordinate values of points on the plane of the cross-sectional area; eigenvalues correspond to proportion of the long axis and the short axis, and eigenvectors correspond to direction of each axis. This means coordinate values of each axis are obtained following equations:

$$\text{the long axis} = \alpha \lambda_1 \vec{x_1} \tag{1}$$

$$\text{the short axis} = \alpha \lambda_2 \vec{x_2} \tag{2}$$

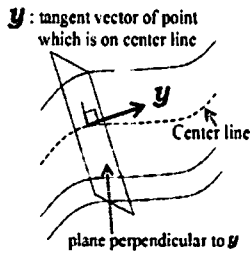$$\alpha = S/\lambda_1 \lambda_2 \tag{3}$$

Fig. 8. The tangent vector and the cross-sectional area

TABLE I

PARAMETERS USED FOR EXPERIMENTS

image resolution	$1.0\ \mu m \times 1.0\ \mu m \times 1.0\ \mu m$
image size	$x = 237, y = 403, z = 110$
branching point	5
distance of minimum each discrete point	10
number of control points of Bezier curve	3

(a) Artificial images

(b) A center line   (c) Discrete points

Fig. 9. Artificial images based on characteristics of a premotor neuron

(a) Skeletons (our previous method)   (b) Median points of ellipses (proposed method)

Fig. 10. Connection of points

where S is cross-sectional area, λ_1 and λ_2 are eigenvalues of a variance-convariance ($\lambda_1 > \lambda_2$), and $\vec{x_1}$ and $\vec{x_2}$ are eigenvectors.

Thirdly, central points, the long axis and the short axis of each central point are grouped with our previous method and they are interpolated with Bezier Curve each branch.

Finally, cross-sectional areas are interpolated using these interpolated parameters of an ellipse.

IV. EXPERIMENT

In this section, the proposed method is applied to an artificial images which is based on characteristics of a premotor neuron, as in Fig. 9(a). Fig. 9(b) shows a result of extraction of a center line through the thinning process. All figures in this section are visualized three-dimensionally using RV-Editor[9].

First, discrete points are selected from Fig. 9(b) and connected with our method for connection of discrete points with Bezier Curves. Secondly, thickness of the form is interpolated using our previous method and the proposed method and each result is compared. In addition, the thickness is interpolated using only a cross-sectional area of a cross-sectional area in discrete points. Finally, the cross-sectional area of the form in each result is compared every branch in order to evaluate the proposed method. Table1 shows parameters in this experiment. Those parameters are determined empirically.

Fig. 10(a) presents the result of connection of points in Fig. 9(c) for every branch. Fig. 11 is the result of interpolation of the distance value of skeletons using our previous method. Though these results show that it is possible to reconstruct a similar form with the artificial images, reconstruction of thicknesses of the form is insufficient in spots.

Fig. 10(b) presents the result of connection of median points, which obtain by cross-sectional area on discrete
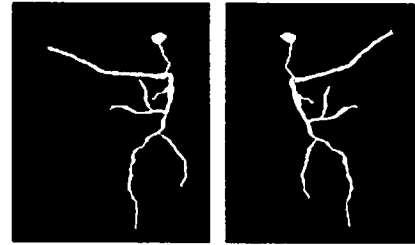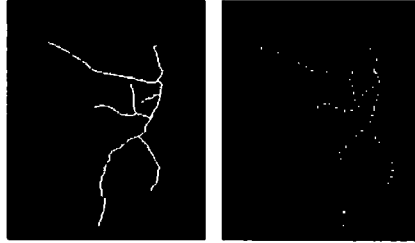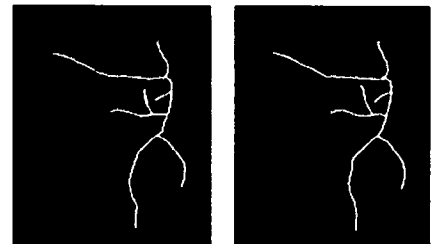
points in Fig. 9(c), for every branch. Fig. 12 is the result of interpolation of cross-sectional area using the proposed method. These results show that it is possible to reconstruct the form which is nearer to the artificial images in comparison with Fig. 11. There are parts which are not reconstructed as like the artificial images. It is thought that a wrong tangent vector of the center line is obtained because of torsion of the center line.

Fig. 13 is each branch of the artificial images. Fig. 14 is comparative result of the cross-sectional area of each interpolation result: a vertical axis is a cross-sectional area of a branch when length of a branch is l ($0 \le l \le 1$) and a horizontal axis is l. Table1I shows mean values of suquare of error cross-sectional areas in the artificial images and each interpolation methods for every branch.

Fig. 14 and Table II show the effect of the proposed method is different by a branch. some cross-sectional areas by proposed method are bigger than results by a distance value of skeletons. However, some cross-sectional areas in results of proposed method are much bigger than the artificial images or smaller than results of our previous method. The

Fig. 11. Interpolation of cross-sectional area using our previous method

Fig. 12. Interpolation of the cross-sectional area using the proposed method

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

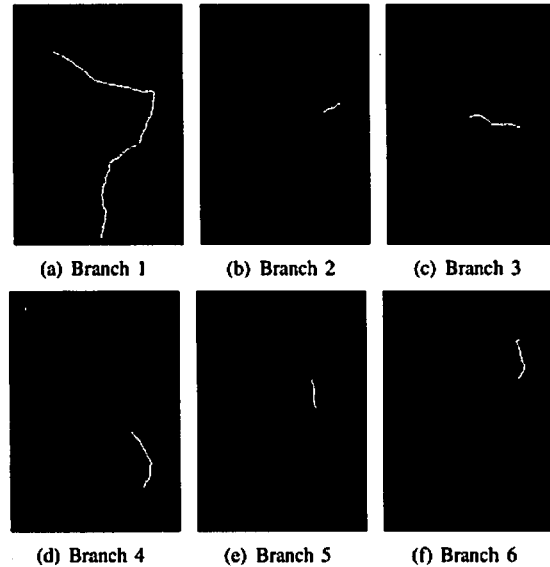(d) Branch 4   (e) Branch 5   (f) Branch 6

Fig. 13. Branches

method using only cross-sectional area interpolates a cross-sectional area smoothly. Then it is thought that this method cannot reconstruct concavity and convexity of the form. On the other hand, the proposed method is able to reconstruct it in some branches because the result of proposed method changes in the same way as the artificial images.

For these results, it is necessary to consider the setting of the tangent vector. The proposed method will be more useful for interpolation of the cross-sectional area of a neuron with a suitable tangent vector.

V. CONCLUSIONS AND FUTURE WORKS

In this paper, we presented a method for interpolation of the cross-sectional area of a neuron using an ellipse model. We have presented a method for interpolation of partial deficiencies of the CLSM binary images to extract the form of a single neuron. Although it is possible to connect partial deficiencies using this method, interpolation of the cross-sectional area is insufficient because this interpolation is with an inscribed circle of a cross-sectional area. In the proposed method, the cross-sectional area is interpolated using an ellipse model. To verify the availability of the proposed method, it was applied to the artificial images series which shows characteristics of the premotor neuron and has no partial deficiencies. Results show that the proposed method is useful for interpolation of cross-sectional areas.

In future works, it is necessary to consider the setting of

TABLE II

ERROR OF MEAN SUQUARE OF CROSS-SECTIONAL AREA IN EACH BRANCH

branch	1	2	3	4	5	6
skeleton	27.22	2.77	132.57	21.92	8.62	2.16
ellipse	45.91	6.40	230.96	38.49	6.85	2.76
cross area	21.14	3.77	61.40	12.83	3.15	1.91

the tangent vector. In addition, we will apply the proposed method to an image series of a premotor neuron captured using CLSM.

REFERENCES

[1] R. Kanzaki, N. Sugi and T. Ahibuya, "Self-generated zigzag turning of Bombyxmori males during pheromone-mediated upwind walking", Zool.Sci., 9, pp. 515-527, 1992.

[2] Y. Seki, H. Aonuma and R. Kanzaki, " Pheromone Processing Center in the Protocerebrum of Bombyxmori Revealed by Nitric Oxide-Induced Anti-cGMP Immunocytochemistry", TheJournalofComparativeNeurology, 480, pp. 340-351, 2005

[3] S. Wada and R. Kanzaki, "Neural Control Mechanisms of the Pheromone-Triggered Programmed Behavior in Male Silkmoths Revealed by Double-Labeling of Descending Interneurons and a Motor Neuron", TheJournalofComparativeNeurology, 484, pp. 168-182, 2005.

[4] K. Nakajima, S. Morishita, T. Kanzawa, R. Kanzaki, H. Asama and T. Mishima, "Compensation of binarized CLSM images for extraction of the form of a single neuron in the silkworm moth brain", IEEEInternationalConferenceonRoboticsandBiomimetics. pp. 1224-1229, 2007.

[5] K. A. Al-Kofahi, S. Lasek, D. H. Szarowski, C. J. Pace, G. Nagy, J. N. Turner and B. Roysam, "Rapid Automated Three-Dimensional Tracing of Neurons from Confocal Image Stacks", IEEETrans.Inform.Techno.Biomed., 6(2), pp. 171-187, 2002.

[6] W. He, T. A. Hamilton, A. R. Cohen, T. J. Holmes, C. Pace, D. H. Szarowski, J. N. Turner and B. Roysam, "Automated Three-Dimensional Tracing of Neurons in Confocal and Brightfield Images", MicroscopyandMicroanalysis, 9, pp. 296-310, 2003.

[7] T. Yamasaki, T. Isokawa, N. Matsui, H. Ikeno and R. Kanzaki, "Reconstruction and Simulation for Three-Dimensional Morphological Structure of Insect Neurons", Neurocomputing, 69, pp. 1043-1047, 2006.

[8] B. Katz, "Nerve, Muscle, and Synapse", McGraw − HillSeriesintheNewBiology.McGraw − Hill, 1966.

[9] T. Saito, K. Mori and J. Toriwaki, "A Sequential Thinning Algorithm for Three Dimensional Digital Picture Using the Euclidean Distance Transformation and Its Properties", TheTransactionsofIEICE, Vol. J79-D-2, No. 10, pp. 1675-1685, 1996.

[10] http://www.riken.go.jp/lab-www/

(a) Branch 1

(b) Branch 2
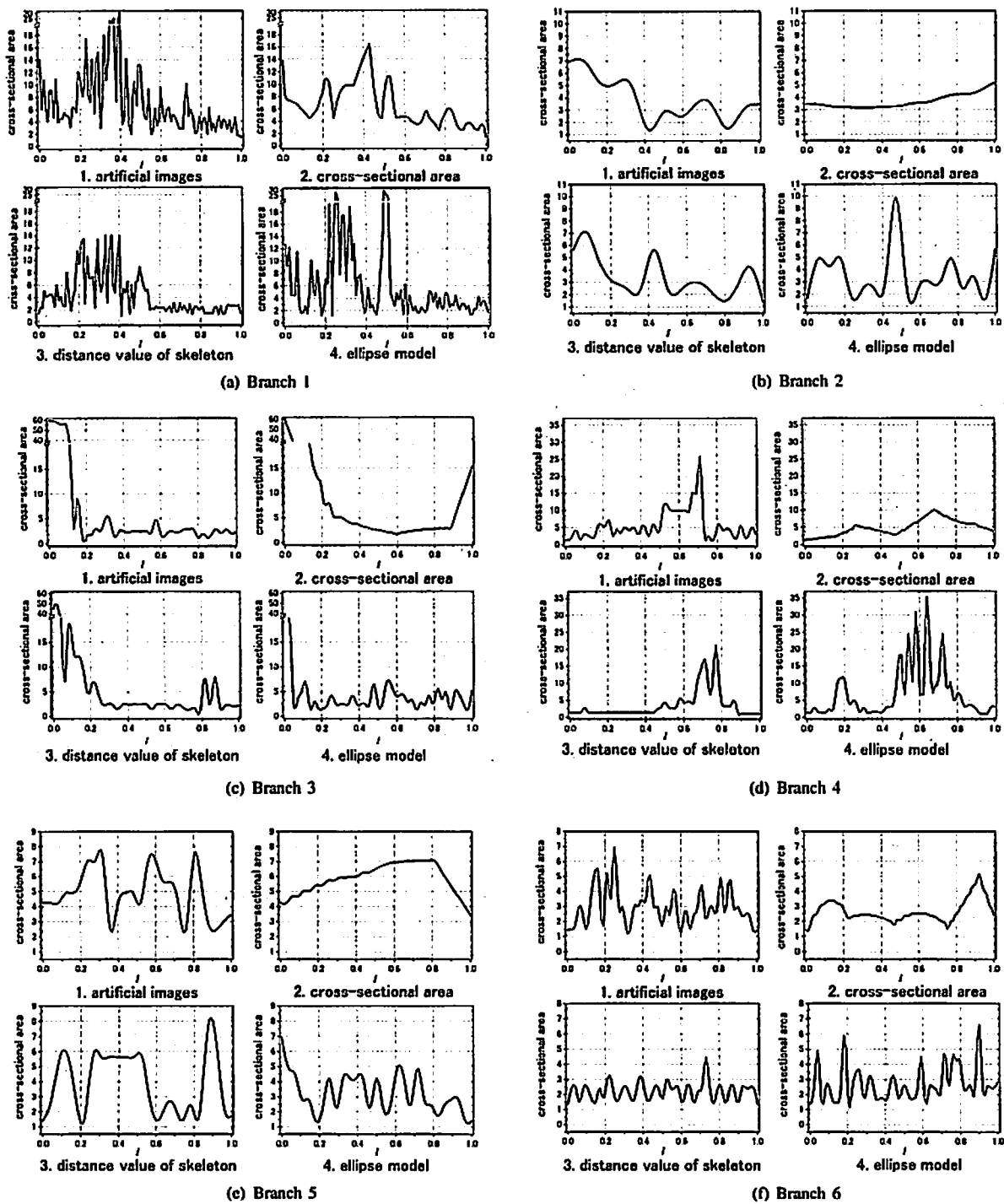
(c) Branch 3

(d) Branch 4

(e) Branch 5

(f) Branch 6

Fig. 14. Results of interpolation of cross-sectional area every branch