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Serially Sectioned and Segmented Images of the Mouse for Learning Mouse Anatomy

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ABSTRACT Mouse anatomy is fundamental knowledge for researchers who perform biomedical experiments with mice. The purpose of our research is to present the serially sectioned images and segmented images of the mouse to produce three-dimensional images of the mouse, which are helpful in learning mouse anatomy.

Using a cryomacrotome, a couple of male and female mice were transversely serially sectioned at 0.5 mm intervals to make sectioned surfaces. The sectioned surfaces were digitalized to make serially sectioned images. In the serially sectioned images of the female mouse, 14 structures including skin and bones were semi-automatically segmented on Adobe Photoshop software to make segmented images. The serially sectioned images and segmented images were stacked to make sagittal and coronal images, which were used for verifying the serially sectioned images and segmented images.

In this ongoing research, the segmented images of male mouse will be added. All serially sectioned images and segmented images of the mouse will be presented worldwide. These images are expected to be used by many researchers for making three-dimensional images and virtual dissection software of the mouse, which are helpful in comprehending the stereoscopic morphology of the mouse's structures.

Key words : Serially sectioned images, Segmented images, Mouse anatomy, Three-dimensional images, Cryomacrotome, Semi-automatic segmentation

INTRODUCTION

Mouse anatomy is fundamental knowledge for researchers who perform biomedical experiments with mice. The mouse anatomy can be learned with a mouse atlas; however, a mouse atlas only has two-dimensional pictures, which is not enough for comprehending the stereoscopic morphology of the mouse's structures. The mouse anatomy can be also learned during dissection of the mouse; however, many mice need to be sacrificed for dissection and the labor is discomforting. If three-dimensional (3D) images of the mouse are made and sectioned at free angles to display the sectioned planes and 3D images of the mouse's structures are rotated at free angles, the stereoscopic morphology of the mouse's structures can be conveniently comprehended. In order to make the 3D images, it is necessary to accompany the serially sectioned images of the mouse with the segmented images of the mouse's structures. However, images such as these haven't been produced yet, although serially sectioned images of the human (Spitzer *et al*., 1996; Park *et al*., 2005a), dog (Böttcher & Maierl, 1999), frog (Nip & Logan, 1991) as well as the mouse embryo (Durikovic *et al*., 1998; Williams & Doyle, 1996) have been made for the same reason.

The purpose of this research is to present the serially sectioned images and segmented images of the mouse to promote 3D images of the mouse, which are helpful in comprehending the mouse anatomy.

In order to achieve this purpose, the following trials were performed. A couple of male and female mice were transversely serially sectioned at 0.5 mm intervals to make sectioned surfaces. The sectioned surfaces were digitalized to make serially sectioned images. In the serially sectioned images of the female mouse, 14 structures including skin and bones were semi-automatically segmented on Adobe Photo-

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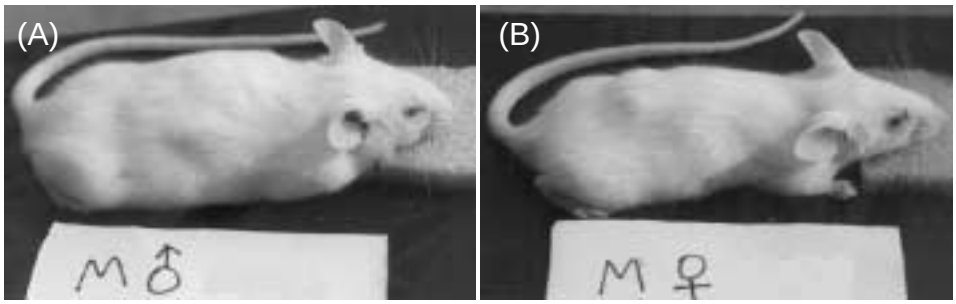

Fig. 1. Male mouse (A) and female mouse (B) selected for this research.

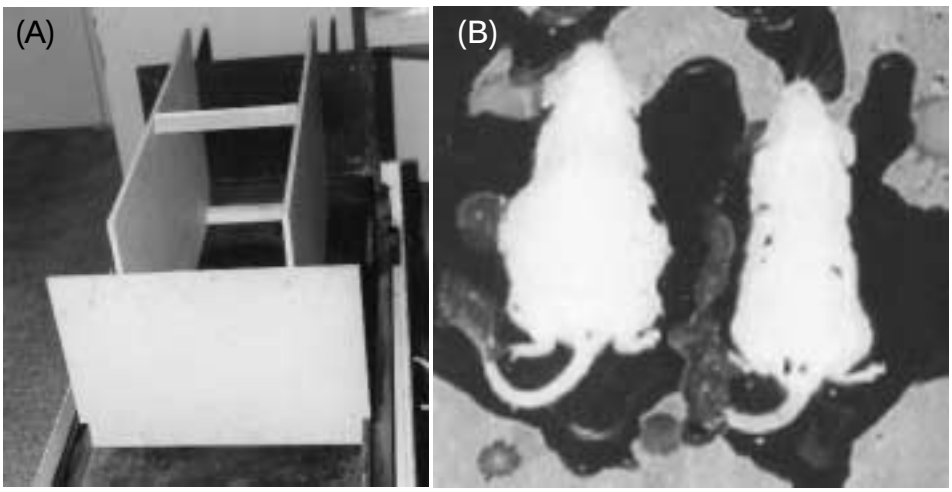

Fig. 2. Embedding box (A), in which male and female mice are placed on the embedding agent base (B).

shop software to make segmented images. The serially sectioned images and segmented images were stacked to make sagittal and coronal images, which were used to verify the serially sectioned images and segmented images.

MATERIALS AND METHODS

A couple of male and female mice (ICR species) were selected and sacrificed. A male mouse (age, 10 weeks old; weight, 39 g; body length, 218.5 mm; body width, 42 mm) and a female mouse (age, 10 weeks old; weight, 29 g; body length, 193.5 mm; body width, 38 mm) were selected for this research. The body length was measured after turning the mice's tails to their body sides (Fig. 1). The mice were sacrificed with diethyl ether.

An embedding box (length, 400 mm; width, 300 mm; height, 500 mm) was made of heavy steel (bottom board and cranial board) and wood (two side boards and caudal board). Upper parts of the two side boards were connected with a wooden rod for preventing the freezing embedding agent from widening the two side boards (Fig. 2A). Blue embedding agent (gelatin, 30 g; methylene blue, 0.5 g; distilled water, 1,000 mL) was poured into the embedding box until the embedding agent filled half of the embedding box, and frozen to −70℃ in a freezer to make the embedding agent base. The surface of the embedding agent base was flattened.

The mice were embedded and frozen in the embedding box in the following methods. Two mice were placed side by side on the embedding agent base. The mice's tails were turned to their body sides; as a result, serial sectioned images including only the mice's tails were reduced to a minimum (Fig. 2B). The mice's heads were directed to the cranial board of the embedding box. The mice were positioned 50 mm away from the cranial board; as a result, serial sectioning of the embedding agent between the cranial board and the mice could be performed as the preliminary experiment. The longitudinal axes of the mice including the spinous processes of the vertebrae were adjusted to be parallel to the longitudinal axis of the embedding box. The embedding agent was fully poured into the embedding box and frozen for making the embedding box heavy.

As the preliminary experiment, the embedding box including only the embedding agent between the cranial board and mice was serially sectioned. The cranial board was detached from the embedding box. The embedding box was serially

sectioned at 0.5 mm intervals (Fig. 3) until a mouse's nose appeared on the sectioned surface. During the preliminary experiment, optimal conditions were decided for making serially sectioned images with good quality: optimal moving speed (20 mm/s) of the milling table was decided; not only optimal rotating speed (628 rpm) of the cutting blade but also optimal quality and angle of the 20 teeth on the cutting blade were decided, and the teeth were replaced with new ones regularly; cold condition of the embedding box for serial sectioning and optimal conditions of the digital camera (DSC 560 Kodak™; resolution, 3,040 × 2,008) and two strobe heads (Digital S, Elinchrom™) for photographing the sectioned surfaces (size, 300 × 200 mm) were decided (Fig. 4A, B) (Park *et al*., 2005a).

As the final experiment, the embedding box including two mice was serially sectioned to make serially sectioned images. The embedding box was serially sectioned at 0.5 mm intervals (Fig. 3). Frost on every sectioned surface was removed with ethyl alcohol. The sectioned surfaces were photographed to make serially sectioned images (pixel size, 0.1 mm) of two mice under constant conditions of the digital camera (F value, 10; shutter speed, 1/250 second; focusing, manual) while the two strobe heads flashed (Fig. 4A, B). The serially sectioned images in the digital camera were transferred to a personal computer. Then the serially sectioned images were saved in tag image file format (TIFF) files. The serial sectioning and photographing processes were repeatedly performed until the mice did not appear on the sectioned surfaces any more (Park *et al*., 2005a).

Fig. 3. Cryomacrotome on which embedding box is serially sectioned by rotating cutting blade to make a sectioned surface.

From the serially sectioned images of two mice (resolution, 3,040 × 2,008), those of the male mouse (resolution, 1,516 × 1,340) and those of the female mouse (resolution, 1,512 × 1,052) were extracted with appropriate margins. Intervals of the serially sectioned images were 0.5 mm. Therefore, in the case of the male mouse (body length, 218.5 mm), 437 serially sectioned images were extracted; in the case of the female mouse (body length, 193.5 mm), 387 serially sectioned images were extracted (Fig. 5A, B; Table 1).

Further experiments were performed only with the serially sectioned images of the female mouse.

Sagittal and coronal images were made of the serially sectioned images. Sagittal images were made of the serially sectioned images (intervals, 0.5 mm; pixel size, 0.1 mm) as follows. As the pre-process, each serially sectioned image was copied and pasted four times between the serially sectioned image and the next serially sectioned image. As a result, we prepared 1,935 fivefold serially sectioned images with 0.1 mm intervals, which were the same as pixel size of the serially sectioned images. Each column of the fivefold serially sectioned images was selected and stacked in sequence on Adobe Photoshop software (version 7.0, Adobe™) to make a sagittal image (Fig. 6A). Repeatedly, 1,512 serial sagittal images were made of the fivefold serially sectioned

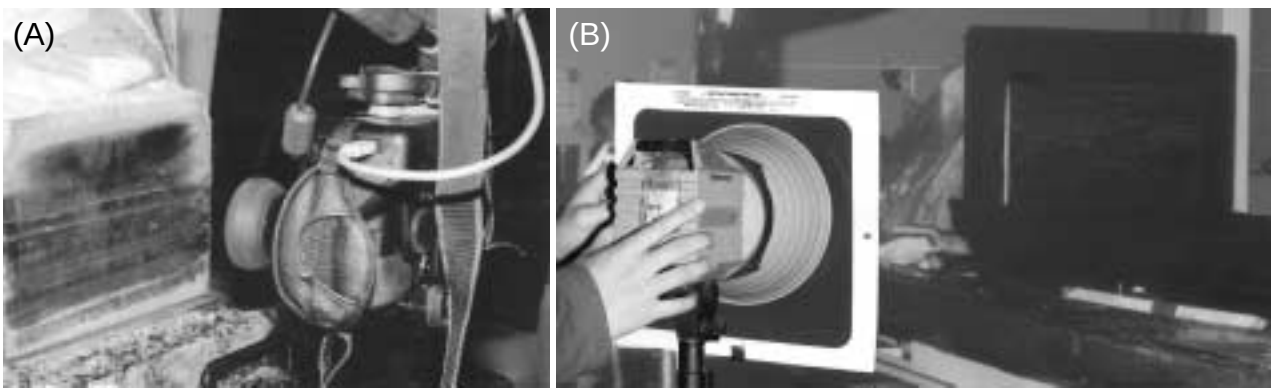

Fig. 4. Digital camera for photographing of the sectioned surfaces (A) and strobe head for flashing on the sectioned surfaces (B).

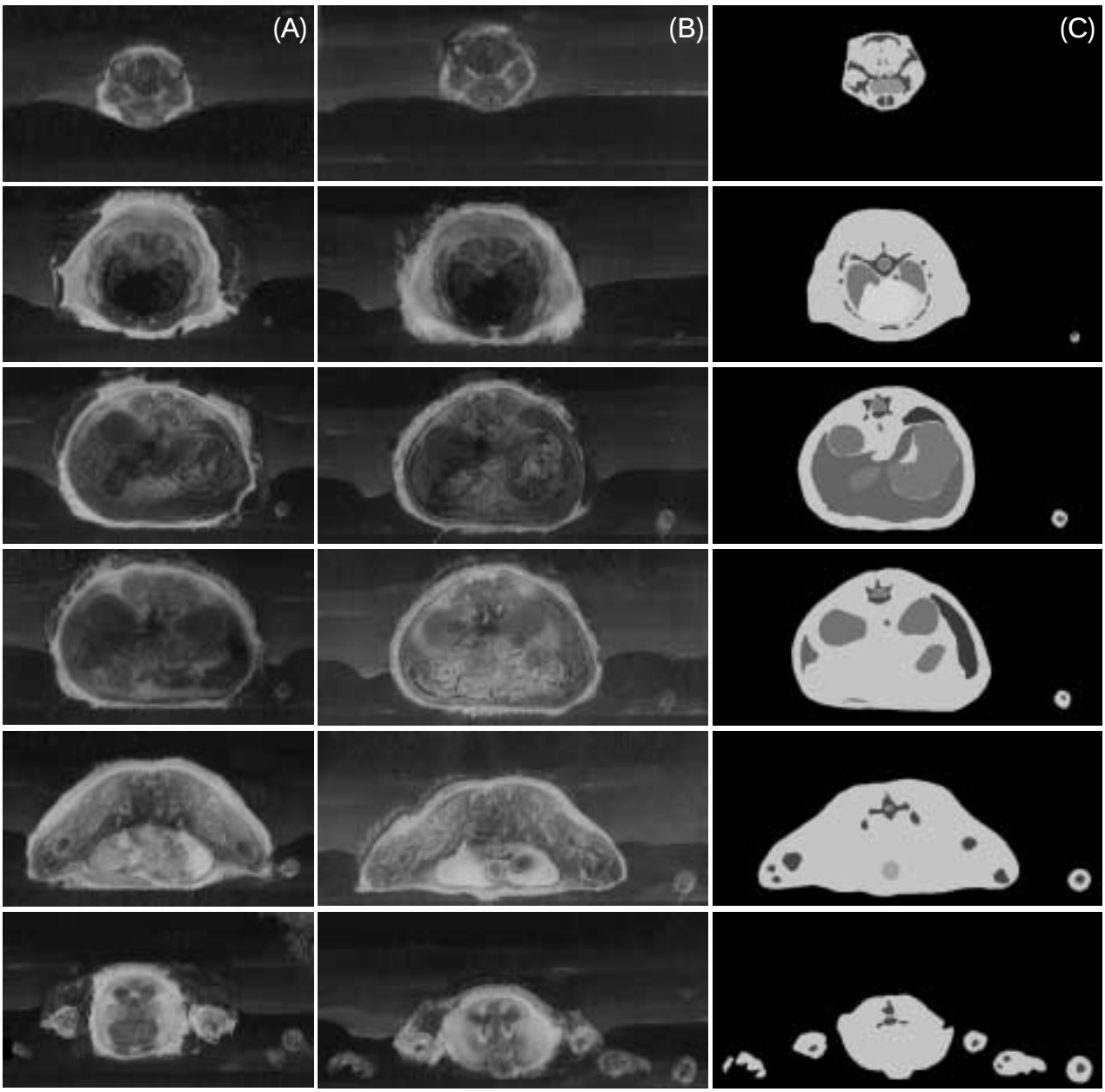

Fig. 5. Serially sectioned images of the male mouse (A), serially sectioned images of the female mouse (B), and segmented images of the female mouse (C).

image. In a similar manner, 1,052 serial coronal images were made from the fivefold serially sectioned image (Fig. 6C) (Park *et al*., 2005b).

The sagittal and coronal images were examined for verifying alignment and constant brightness of the serially sectioned images. In some sagittal and coronal images, smoothness of each structure's contour was examined for verifying alignment of the serially sectioned images. In the same images, brightness of the structures was examined for verifying constant brightness of the serially sectioned images (Fig. 6A, C). If either incorrect alignment or inconsistent brightness was found, the serially sectioned images were revised on Adobe Photoshop software.

Fourteen structures (skin, bones, digestive tract, liver, gallbladder, respiratory tract, lungs, heart, aortas, kidneys, urinary bladder, brain, spinal cord, and spleen) were decided to be segmented in the serially sectioned images because the structures were not only important but also relatively distinct in the serially sectioned images.

Fourteen structures were semi-automatically segmented on

Table 1. Features of the serially sectioned images and segmented images of the mice

Sex	Images	Number	Resolution	Bits depth (Bits color)	One file size (MBytes)	Total file size (GBytes)
Male	Serially sectioned	437	1,516 × 1,340	24	5.9	2.5
Female	Serially sectioned	387	1,512 × 1,052	24	4.6	1.7
Female	Segmented	387	1,512 × 1,052	8	1.5	0.5

Intervals, 0.5 mm; Pixel size, 0.1 mm; File format, Tag image file format (TIFF)

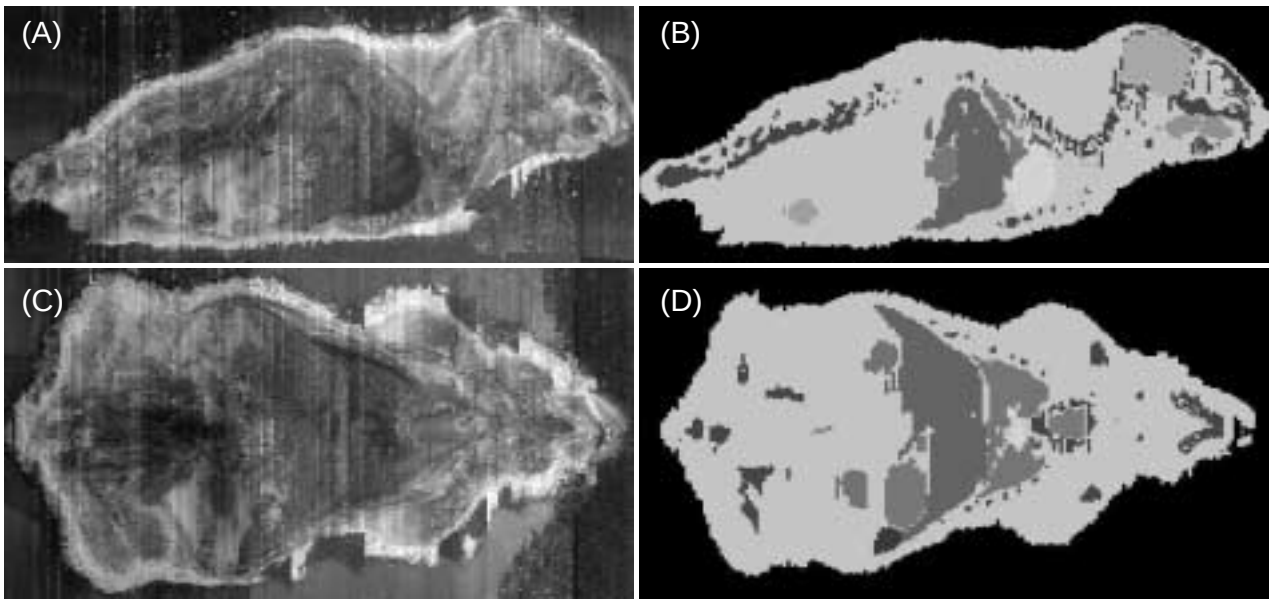

Fig. 6. Sagittal serially sectioned image (A), sagittal segmented image (B), coronal serially sectioned image (C), and coronal segmented image (D) of the female mouse.

Adobe Photoshop software to make segmented images. Contours of 14 structures were identified in all 387 serially sectioned images by referring to the serial slices atlas of the mouse (Iwaki *et al*., 2001). The contour of each structure was semi-automatically drawn using magnetic lasso tool of Adobe Photoshop software; subsequently, the contour was automatically filled with a specific segmentation color to make the 387 segmented images (Park *et al*., 2005b). Resolution (1,512 × 1,052), pixel size (0.1 mm), and file format (TIFF) of the segmented images were adjusted to be the same as those of the serially sectioned images. However, bits depth of the segmented images was adjusted to be 8 bits color, namely 2^8 (=256) kinds of colors, which were enough for representing 14 segmentation colors (Fig. 5C; Table 1).

The sagittal and coronal images were made and examined for verifying correct segmentation. Sagittal and coronal images of the segmented images were made in the same manner as those images of the serially sectioned images. In some sagittal and coronal images, it was examined whether each structure's contour was smooth and fit to anatomical knowledge, which meant that correct segmentation was done (Fig. 6B, D). If segmentation was incorrect, the segmented images were revised on Adobe Photoshop software.

RESULTS

From a male mouse, 437 serially sectioned images (TIFF files) with 0.5 mm intervals were acquired. Each serially sectioned image had 1,516 × 1,340 resolution, 24 bits color, and 5.9 MBytes file size; total serially sectioned images had 2.5 GBytes file size (Table 1).

From a female mouse, 387 pairs of serially sectioned images and segmented images (TIFF files) with 0.5 mm intervals were acquired. Each serially sectioned image had 1,512 × 1,052 resolution, 24 bits color, and 4.6 MBytes file size; total serially sectioned images had 1.7 GBytes. Each segmented image had 1,512 × 1,052 resolution, 8 bits color, and 1.5 MBytes file size; total segmented images had 0.5 GBytes file size (Table 1).

The serially sectioned images were acquired with good quality. The serially sectioned images had 0.1 mm pixel size, so that structures greater than 0.1 mm could be identified in the serially sectioned images; in addition, the serially sectioned images showed colors similar to the living mouse. But the serially sectioned images did not show the mouse's hairs in nature (Fig. 5A, B). The serially sectioned images were aligned and had constant brightness in general, which was verified by examining the sagittal and coronal images (Fig. 6A, C).

Segmented images were acquired with good quality as well. The segmented images corresponding to the serially sectioned images showed apparent contours of the 14 structures (Fig. 5C). The segmented images were generally correct, which was verified by examining the sagittal and coronal images (Fig. 6B, D).

DISCUSSION

The serially sectioned images of the mouse need to be made. After staking the serially sectioned images, 3D image can be made by volume-reconstruction. The 3D image can be sectioned at free angles to display the sectional planes. The 3D image is helpful in comprehending stereoscopic morphology (Pommert *et al*., 2001; Schiemann *et al*., 2002). For comprehending stereoscopic morphology of the various animals, the serially sectioned images of the dog (Böttcher & Maierl, 1999), frog (Nip & Logan, 1991), and mouse embryo (Williams & Doyle, 1996; Durikovic *et al*., 1998) were made. But the serially sectioned images of the mouse, which is a widely used experimental animal, were not produced yet. Therefore, in this research, serially sectioned images of the mouse were decided to acquire.

The serially sectioned images of the mouse need to be made by the following principles.

First, the serially sectioned images need to be transverse, parallel to each other, and they need to have constant intervals. If not, the 3D image made of the serially sectioned images becomes distorted. Serially sectioned images of the dog were not produced in these conditions because the embedding box was not firmly fixed on the milling table of the cryomacrotome and the milling table was manually moved (Böttcher & Maierl, 1999). The serially sectioned images of the frog were not produced in these conditions too because the cryomacrotome was frequently jammed during serial sectioning (Nip & Logan, 1991). In this research, for making the serially sectioned images transverse, surface of the embedding agent base was flattened and all longitudinal axes of the mouse, embedding box, and milling table were adjusted to be parallel. To make the serially sectioned images parallel to each other and their intervals constant, the reliable cryomacrotome, whose moving error was just 1 μm, was made; the embedding box, which was made as heavily as possible, was placed on a constant place of the milling table and firmly fixed; serial sectioning of the embedding box was repeatedly performed without human error by a computer program (Figs. 2, 3).

Second, the serially sectioned images need to be aligned. If not, the 3D image becomes distorted too. Serially sectioned images of the mouse embryo were not produced in this condition because the serially sectioned images were made by photographing with film camera and by scanning the films with laser slide scanner, which might cause incorrect alignment (Williams & Doyle, 1996; Durikovic *et al*., 1998). In this research, to make the serially sectioned images aligned, every sectioned surface was moved to a constant position for photographing; the location and direction of the digital camera were always fixed (Fig. 4A); and alignment of the serially sectioned images was verified by examining the sagittal and coronal images (Fig. 6A, C).

Third, the serially sectioned images need to have constant brightness. If not, the 3D image can not yield sectional planes with good quality. Serially sectioned images of the frog were not produced in this condition because daylight from laboratory windows influenced the sectioned surfaces during photographing (Nip & Logan, 1991). In this research, to make the serially sectioned images with constant brightness, the laboratory was made dark; the location and direction of the two strobe heads were adjusted and always fixed (Fig. 4B); the F value and shutter speed of the digital camera were always fixed; and the constant brightness of the serially sectioned images was verified by examining the sagittal and coronal images (Fig. 6A, C).

Fourth, the serially sectioned images need to involve the sectioned surfaces with good quality. If the sectioned surfaces don't keep good quality, the corresponding serially sectioned images can not show apparent structures. In this research, to make the sectioned surfaces with good quality, the milling table was moved and the cutting blade was rotated at optimal speed (Fig. 3); the teeth with optimal quality were mounted on the cutting blade at optimal angle and replaced with new ones regularly; the embedding box was firmly fixed on the milling table and hard frozen during serial sectioning; and frost on the sectioned surfaces was removed.

Fifth, the serially sectioned images need to have the pixel size as small as possible. If not, small structures can not be identified in the serially sectioned images. In this research, to

make the serially sectioned images have 0.1 mm pixel size, sectioned surfaces (size, 300 × 200 mm) were photographed using the digital camera (resolution, 3,040 × 2,008). However, the serially sectioned images had 0.5 mm intervals, so structures smaller than 0.5 mm may not appear in the serially sectioned images (Table 1). In the next research, intervals of the serially sectioned images need to be reduced.

Segmented images of the mouse need to be made. After stacking the segmented images, 3D image of each structure can be made by volume-reconstruction or surface-reconstruction. The 3D images of such structures can be selected to be displayed and rotated at free angles. The 3D images are helpful in comprehending the stereoscopic shape and locational relationship of structures (Pommert *et al*., 2001; Schiemann *et al*., 2002). Therefore, in this research, the segmented images of the mouse's important structures including skin and bones were decided to acquire.

The segmented images of the mouse need to be made by the following principles.

First, the structures' contours need to be semi-automatically drawn. If the contours are manually drawn, segmentation would require tedious work and long time; and segmentation is not guaranteed to be objective (Nip & Logan, 1991). Reversely, if the contours are automatically drawn, segmentation itself would not be possible because most structures in the serially sectioned images can not be identified by the computer but only by a human. In this research, the contours of the mouse's structures were semi-automatically drawn on Adobe Photoshop. Magnetic lasso tool of Adobe Photoshop was adequate enough for semi-automatic segmentation that specific software for segmentation was not necessary (Park *et al*., 2005b).

Second, the segmentation needs to be verified. Segmentation errors may occur while various kinds of structures are segmented in a lot of serially sectioned images. If the segmentation is not verified, the 3D image of each structure may become distorted. It is difficult to find the incorrect segmented images by examining the segmented images themselves. In this research, segmented images of the mouse's structures were verified by examining the sagittal and coronal segmented images (Fig. 6B, D), and incorrect segmented images were revised (Park *et al*., 2005b).

In this research, 437 serially sectioned images of a male mouse as well as 387 pairs of serially sectioned images and segmented images of a female mouse were made (Fig. 5A, B, C; Table 1). The segmented images of the male mouse will be added. All serially sectioned images and segmented images of the mouse will be presented worldwide. These images are expected to be used by many researchers for making 3D images and virtual dissection software of the mouse, which are helpful in comprehending the stereoscopic morphology of the mouse.

Upgraded data of the serially sectioned images and segmented images will be made in the near future as follows. The serially sectioned images of the mouse with thinner intervals will be made; the detailed segmented images of the mouse will be made; the computed tomographs and magnetic resonance images of the mouse with high resolution will be made; the serially sectioned images and segmented images of other experimental animals (e.g. rat, rabbit, and so on) will be made.

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생쥐의 해부학을 익히기 위한 생쥐의 연속절단면영상과 구역화영상

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초 록 : 생쥐를 갖고 실험하는 연구자는 생쥐의 해부학을 알아야 한다. 생쥐의 연속절단면영상과 구역화영상을 쌓아서 3차원영상을 만들면 생쥐의 입체 생김새를 이해하는 데 도움 될 것이다. 이 연구의 목적은 생쥐의 연속절단면영상과 구역화영상을 만들고 이를 널리 퍼뜨려서 다른 연구자가 생쥐의 3차원영상을 만들게 하는 것이다.

연속절단기를 써서 수컷 생쥐와 암컷 생쥐를 0.5 mm 간격으로 가로절단한 다음에 절단면을 디지털사진기로 찍어서 연속절단면영상을 만들었다. 암컷 생쥐의 연속절단면영상에서 보이는 14개 구조물을 반자동으로 구역화해서 (어도비 포토샵 소프트웨어) 구역화영상을 만들었다. 연속절단면영상과 구역화영상을 쌓아서 이마, 마루영상을 만든 다음에 정렬이 맞는지, 구역화가 제대로 되었는지 확인하였다.

앞으로 수컷 생쥐의 구역화영상도 만들 계획이다. 이 연구에서 만든 생쥐의 연속절단면영상과 구역화영상을 퍼뜨리면, 다른 연구자가 생쥐의 3차원영상과 가상해부 소프트웨어를 만들 수 있고, 나아가 생쥐의 입체 생김새를 이해하는 데 도움 될 것이다.

찾아보기 낱말 : 연속절단면영상, 구역화영상, 생쥐 해부학, 3차원영상, 연속절단기, 반자동 구역화