Three-dimensional cellular and subcellular structures of human brain tissue determined by microtomography

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Abstract. We report here x-ray microtomographic studies of human cerebral cortex stained with high-Z elements. Brain tissues were stained with metal elements by the Golgi and Bodian impregnation methods and subjected to x-ray microtomographic analysis. Axons and dendrites arising from cell bodies were visualized as three-dimensional networks. Spherical structures of cellular nuclei were observed in the interiors of cell bodies, indicating that hard x-ray microtomography can reveal the intracellular structure. High-Z element microcontrasting in conjunction with microtomographic analysis can be applied to any soft tissues. Our results show that the metal contrasting facilitates the three-dimensional microtomographic visualization of cellular and subcellular structures of soft tissues.

1. Introduction
The transparency of biological tissue to hard x-rays enables radiographic analysis of the internal structure. However, soft tissues are composed of light elements, which produce little contrast in a hard x-ray transmission image. In clinical diagnosis, luminal structures of a living body are visualized by using x-ray contrast media. These contrast media contain high-Z elements, such as barium or iodine, that absorb x-rays efficiently. We have recently reported the three-dimensional visualization of the soft tissue microstructure of fruit fly [1,2], zebrafish [3], and human brain [3,4] by contrasting with high-Z elements. Element-specific structural analysis has also been achieved by using x-ray absorption edges of the contrasting elements [5].

Brain functions including creation, memory, and emotion are performed by three-dimensional neuronal networks composed of a huge number of neurons. The cellular functions of each neuron are achieved by the intracellular structure of neurons. Therefore, three-dimensional microstructural analysis of nerve tissues should reveal the functional mechanism of the brain. Here, we report on a computed microtomography (micro CT) analysis of the three-dimensional structure of human cerebral cortex at subcellular resolution.
2. Materials and Methods

2.1. Human brain tissue
Frontal cortex of normal brain tissue (44 years old, male) was dissected at autopsy and fixed with 10% formaldehyde for 7 days.

Golgi impregnation was performed using fixed tissues with dimensions of about 5 mm × 5 mm × 10 mm, as described previously [4]. The chromate and silver nitrate steps were repeated three times.

Bodian impregnation was performed using fixed tissues with typical dimensions of 0.5 mm × 0.5 mm × 2.5 mm. Defatting was performed by immersing the dehydrated tissues sequentially in acetone for 24 hr and xylene for 24 hr. After being rehydrated, tissues were subjected to reduced-silver Bodian impregnation, as described previously [2,3].

2.2. Micro CT
Stained samples were embedded in epoxy resin, as described previously [4]. Micro-CT analysis was performed at the BL20XU beamline [6] of SPring-8. Transmission radiographs were recorded with a CCD-based x-ray imaging detector (AA50 and C4880-41S, Hamamatsu Photonics, Japan) using 12.000-keV x-rays. The field of view and effective pixel size of the image detector were 1.00 mm × 0.65 mm and 0.50 μm × 0.50 μm, respectively. A total of 1800 images were acquired with a rotation step of 0.10° and an exposure time of 300 ms. The spatial resolution of the three-dimensional structure was estimated to be 1.0 μm. The convolution back projection method using a Chesler-type filter was used for tomographic reconstruction [7]. Volume-rendered figures of the obtained three-dimensional structures were produced using the program VG Studio MAX (Volume Graphics, Germany). CT densities were rendered by the maximum projection method.

3. Results and Discussion
Golgi impregnation is a conventional staining method for the optical observation of neural cells [8]. It has been reported that only a limited population of neurons is stained with this method. A three-dimensional structure of the internal pyramidal layer of the frontal cortex tissue is shown in Figure 1. Neurons were visualized as a three-dimensional distribution of linear absorption coefficients. Axons and dendrites arise from cell bodies and form network structures. Spherical structures were seen in the interiors of the cell bodies, indicating that the micro-CT analysis can reveal the intracellular microstructure. These microstructures were assigned as cellular nuclei. The surroundings of nuclei were stained deeper than the cytoplasm, while some of the nuclei displaced the metal dye and showed negatively stained images. Blood capillary vessels with an approximate diameter of 6–8 μm were observed as luminal structures, in which blood cells aligned along the lumen [3].

Reduced-silver Bodian impregnation is another conventional method used for the optical observation of neural tissues [9]. This method stains every neuropils with gold [2]. A layer of the three-dimensional structure of human frontal cortex stained with Bodian impregnation is shown in Figure 2. Spherical structures along with neuropil networks can be seen. These spherical structures have diameters of approximately 4–6 μm and should be cellular nuclei of smaller neural cells. Within these spherical structures, even denser granules having a typical dimension of 1.5 μm, were observed. This image suggests that the nucleolus can be visualized by this method.

4. Conclusion
High-Z element microcontrasting in conjunction with micro-CT analysis can be applied to any soft tissues. Our results indicate that the metal contrasting facilitates the three-dimensional visualization of cellular and subcellular structures.

The neuronal network visualized in the three-dimensional structure is essential for cerebral functions. The brain model should be built up as a circuit to reveal its functions, so it is essential to trace neuronal tracts in order to understand the functional mechanism of the brain.
Figure 1. Three-dimensional structure of human frontal cortex stained using Golgi impregnation. CT densities are rendered from linear absorption coefficients of 0 to 180 cm\(^{-1}\). Scale bar: 50 µm.

Figure 2. Three-dimensional structure of human frontal cortex stained using Bodian impregnation. CT densities are rendered from linear absorption coefficients of 0 to 40 cm\(^{-1}\). Scale bar: 20 µm.

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