

## Revealing Human Brain Circuits by Microtomography

The three-dimensional cellular and subcellular structures of biological tissues and organs are essential for their functions. This particularly holds true with the brain. Neurons build up neuronal circuits as three-dimensional networks in the brain tissue. Human brain functions, including verbal ability and scientific thinking that you are now exerting to read this report, solely depend on neuronal circuits. Therefore, visualizing the three-dimensional neuronal networks of the brain tissue is the first step to understanding the functional mechanisms of the human brain.

Computed tomography (CT) is a noninvasive technique for visualizing visceral structures. The application of X-ray microscopy approaches to CT analysis has led to the visualization of three-dimensional microstructures, although biological samples composed of light elements give little contrast in hard X-ray images. Although the application of phase contrast techniques to unstained biological tissues delineated the distribution of native electron densities, the electron density itself has no inherent relationship with biological functions or cellular organization.

In clinical diagnosis, luminal structures of a living body are visualized using X-ray contrast media. These contrast media contain high-atomic-number (high-Z) elements that absorb hard X-rays efficiently. Therefore, the X-ray visualization of microstructures of soft tissues can be performed by contrasting each biological constituent with high-Z probes, which corresponds to fluorescent labels in light microscopy. Labelling with high-Z probes allows radiographic analysis of the three-dimensional structure of neural tissues [1]. The distributions of multiple high-Z probes can be individually visualized using the X-ray absorption edge of each probe element [2].

Staining neurons with a high-Z probe facilitates the three-dimensional structural study of neuronal networks. We have recently reported the three-dimensional microstructural analysis of human brain tissue using microtomographs [3]. Human neuronal networks were clearly revealed in the microtomographic image of the brain tissue stained with the high-Z probe. Therefore, microtomographic analysis leads to the revealing of the neuronal circuits responsible for human brain functions (Fig. 1).

Human samples were obtained with informed consent, using protocols approved by the clinical

research review board. Normal brain tissue from the middle part of the inferior frontal gyrus was dissected at autopsy. The tissue was stained and embedded as described previously [2-4]. Microtomographic analyses were performed at **BL20XU**, **BL20B2** and **BL47XU** beamlines. Radiographs taken by rotational scan were subjected to CT reconstruction using the program RecView (available from <http://www.el.u-tokai.ac.jp/ryuta/>) accelerated with CUDA parallel-computing processors. The spatial resolutions of the microtomographs were estimated using three-dimensional test patterns [e.g. Ref. 5].

The obtained structure indicated that the neuronal networks and capillary vessel architectures were visualized as the three-dimensional distribution of X-ray absorption coefficients. Skeletonized wire models of the structural constituents were built by placing and connecting nodes in the three-dimensional density maps. The model-building procedures were similar to those reported for crystallographic analyses of macromolecular structures, while neuronal processes were automatically traced using a three-dimensional Sobel filter [4]. The cell types of the models were determined from the morphology of somata and dendrites. Electron density maps and a skeletonized wire model of pyramidal neuron 3013 in Layer III of the gray matter are shown in Fig. 1(b).

The three-dimensional structure of the obtained models clarified the network structures embedded in the brain tissue (Fig. 1(c)). The neuronal circuits were then resolved from the neuronal models. The analytical approach based on the three-dimensional structure of the neuronal networks allows discussions of the operating mechanism of the neuronal circuits in the human frontal cortex [4].

At present, the primary method for visualizing three-dimensional structures of soft tissues including neural tissue is confocal light microscopy. However, the absorptive and refractile nature of ultraviolet, visible and infrared lights with respect to biological tissues makes it difficult to visualize three-dimensional structures of block samples. Results obtained in our studies indicated that the three-dimensional microstructure of human brain tissue can be visualized by using the microtomographs. Therefore, the synchrotron radiation microtomography along with high-Z probe contrasting is a potential method of revealing the neuronal circuits of brain, like X-ray crystallography in molecular biology.

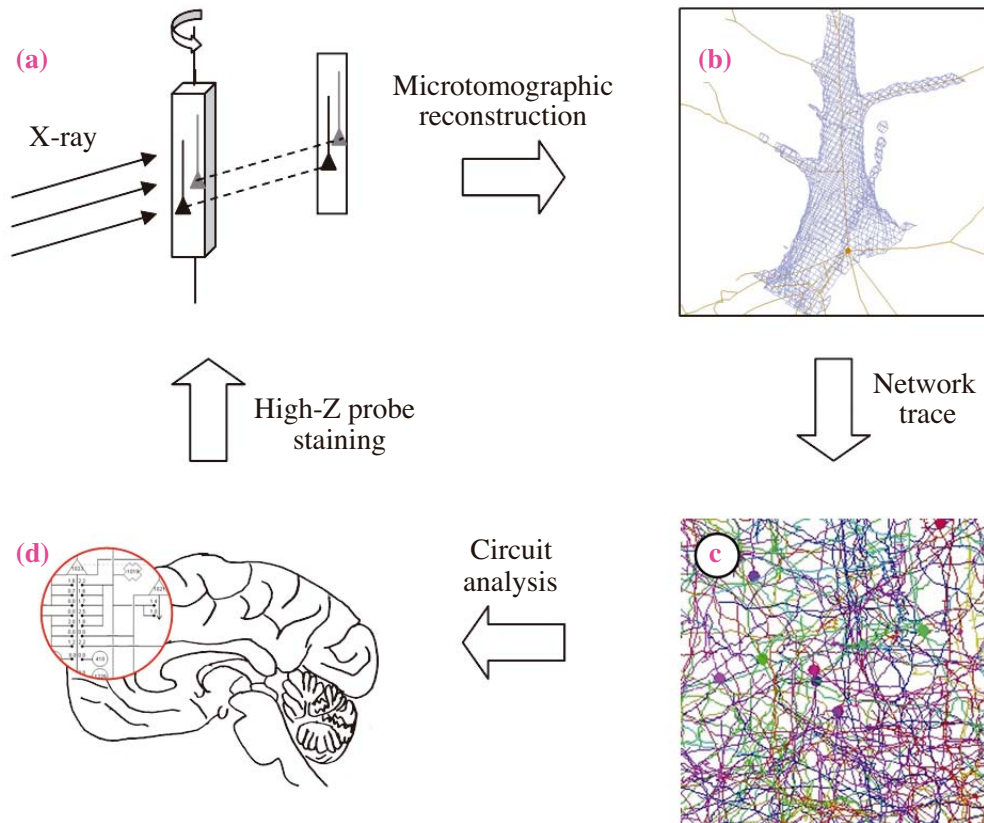


Fig. 1. Schematic diagram of microtomographic analysis of neuronal circuits in human brain. Human brain tissue dissected at autopsy was subjected to high-Z probe staining. The three-dimensional microstructure of the brain tissue was determined by microtomographic analysis (a). The obtained three-dimensional maps were used for density tracing (b), giving the neuronal networks (c). Neuronal circuits responsible for human brain functions were analytically resolved from the traced networks (d).

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#### References

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