Three-Dimensional Neuronal Structure of Human Cerebral Cortex Determined by Synchrotron-Radiation Microtomography

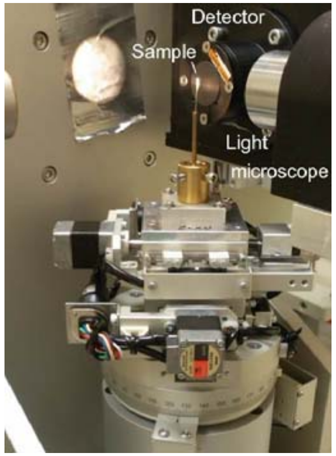
Rino Saiga[1], Susumu Takekoshi[2], Chie Inomoto[2], Naoya Nakamura[2], Akio Tsuboi[2], Motoki Osawa[2], Makoto Arai[3], Kenichi Oshima[3], Masanari Itokawa[3], Kentaro Uesugi[4], Akihisa Takeuchi[4], Yasuko Terada[4], Yoshio Suzuki[4] and Ryuta Mizutani*[1]

[1.] Department of Applied Biochemistry, Tokai University; [2.] Tokai University School of Medicine; [3.] Tokyo Metropolitan Institute of Medical Science; [4.] Japan Synchrotron Radiation Research Institute (JASRI/SPring-8)

*ryuta@tokai-u.jp

X-ray microtomographic analysis of brain network

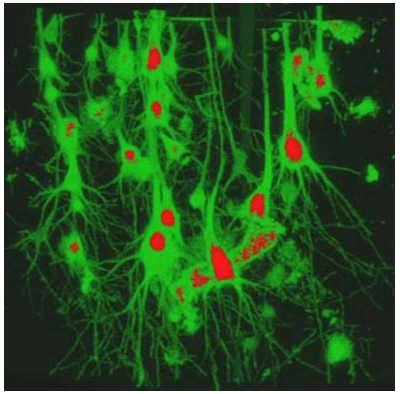
Neuronal circuits are responsible for brain functions including cognition, reasoning, and decision making. Neurons constitute neuronal circuits by forming three-dimensional networks in the cerebral cortex tissue. Therefore, the functional mechanism of human brain can be revealed by visualizing and analyzing the three-dimensional structure of the cerebral cortex. Here, we report on the three-dimensional structure of human cerebral-cortex tissue, as determined by synchrotron-radiation microtomography at resolutions up to 100 nm [1-3].



←X-ray microtomography at SPring-8

Post-mortem cerebral tissues were collected with informed consent from the legal next of kin using protocols approved by ethical committees of the related organizations. The cerebral tissues were subjected to Golgi impregnation, as described previously [4-6].

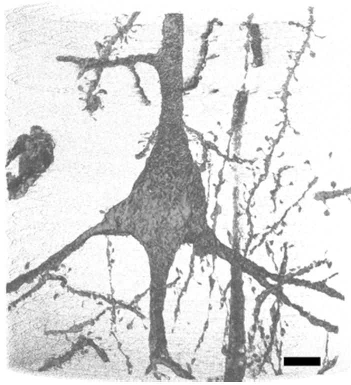
Simple projection x-ray microtomography was performed at the BL20XU beamline of the SPring-8 synchrotron radiation facility. Transmission images produced by 12-keV X-rays were recorded using a CMOS-based detector. A total of 1800 images per dataset were acquired with a rotation step of 0.10º and exposure time of 150 ms per image.



Structure of human frontal cortex ↑

Axons and dendrites arising from somas were visualized as network structures. Cellular nuclei were seen in the interior of somas, indicating that the microtomographic analysis can reveal the subcellular structures.

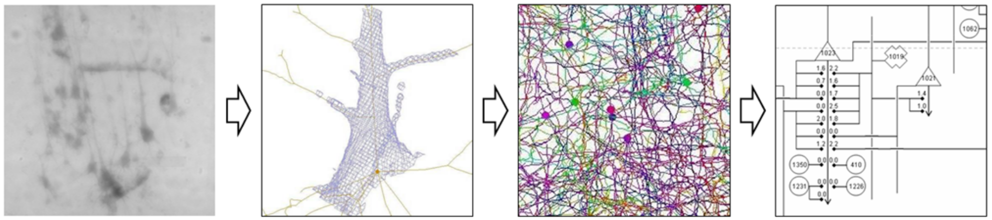
The obtained image illustrated complicated neuronal networks that cannot be comprehended at a glance. As a result, the image had to be further analyzed to reveal the neuronal circuits embedded in the cerebral tissue. Such neuronal network models can be built by placing and connecting nodes in the three-dimensional map of x-ray attenuation coefficients that represent electron densities.



Structure at 100 nm resolution ↑

X-ray microtomography equipped with Fresnel zone plate (FZP) optics was performed at the BL37XU beamline of SPring-8. Spatial resolution was estimated to be 100 nm by using test objects [7,8].

Dendritic spines responsible for neuro-transmission were observed as small protruding structures from the dendrites. These spines have claviform heads with longitudinal lengths of 300-950 nm and cross-sectional diameters of 150-350 nm. Scale bar: 5.0 um.



X-ray image ⇨ Attenuation coeff. map ⇨ Neuronal coordinates ⇨ Brain circuits

Skeletonized model building

First, the neuronal networks and capillary vessel architectures in the cerebral cortex tissue were visualized as a three-dimensional distribution of x-ray attenuation coefficients. Skeletonized wire models of the structural constituents were built by placing and connecting nodes in the coefficient map. The model-building procedures were similar to those reported for macromolecular crystallography, while neuronal processes were automatically traced by using Sobel filter and gradient vector flow method. The neuronal circuits were then analytically resolved from the wire model. This analytical approach based on the structure of the neuronal networks allows discussion of the operating mechanism of the neuronal circuits in the human brain [1-3].

[1] R. Mizutani *et al*. (2010). *Cerebral Cortex* **20**, 1739-1748.
[2] R. Mizutani, R. Saiga *et al*. (2013). *J. Struct. Biol.* **184**, 271-279.
[3] R. Mizutani *et al*. (2011). *AIP Conf. Proc.* **1365**, 403-406.
[4] R. Mizutani *et al*. (2008). *Brain Res.* **1199**, 53-61.
[5] R. Mizutani *et al*. (2008). *Tissue Eng. C* **14,** 359-363.
[6] R. Mizutani & Y. Suzuki (2012). *Micron* **43**, 104-115.
[7] R. Mizutani *et al*. (2010). *Micron* **41,** 90-95.
[8] R. Mizutani *et al*. (2010). *Nuclear Instrum. Meth. A* **621,** 615-619.

Human brain networks→

Neurons are color coded. Closed circles indicate soma coordinates. Possible synaptic connections are indicated with black dots. The brain surface is to the top. Total width: 240 um.

Although the skeletonized model looks complicated, any neurons can be extracted from the model (upper right). Since the models are described in terms of three-dimensional Cartesian coordinates, the distances between the neuronal processes or somas can be directly calculated from the coordinates. This enabled us to determine individual neuronal circuits by analyzing the positional relationships of the neurons [1].

The pair of neurons shown upper right connects their inputs and outputs to each other to form a feedback loop (lower right). In electronics, a similar feedback circuit composed of transistors is known as an astable multivibrator, which generates a string of pulses. Since such feedback loops are formed if a number of neurons are connected to each other, the loop circuit should be one of the canonical structures of human brain circuits.