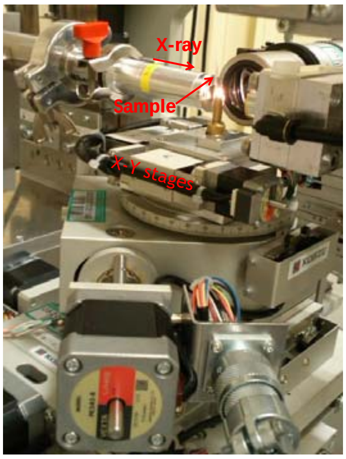
Synchrotron Radiation Nanotomography of Biological Soft Tissues

Rino Saiga[1], Susumu Takekoshi[2], Chie Inomoto[2], Naoya Nakamura[2], Makoto Arai[3], Kenichi Oshima[3], Masanari Itokawa[3], Masato Hoshino[4], Akihisa Takeuchi[4], Kentaro Uesugi[4], Yasuko Terada[4], Yoshio Suzuki[5] and Ryuta Mizutani[1]*

[1]Tokai Univ; [2]Tokai Univ Sch of Medicine; [3]Tokyo Metropolitan Institute of Medical Sci; [4]JASRI/SPring-8; [5]Univ of Tokyo; *ryuta@tokai.ac.jp

X-ray nanotomography of biological soft tissues

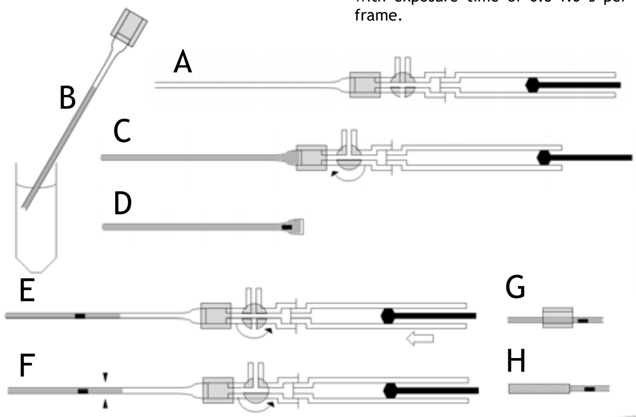
Biological soft tissues are composed of micrometer- to nanometer-scale structures of cellular and subcellular constituents. In order to visualize tissue structures with nanotomography (nano-CT), each constituent must keep its structure constant at nanometer precision throughout the image acquisition. Since soft tissues are easily deformed, samples should be pretreated taking account of a number of factors that affect the image. Here, we report the application of synchrotron radiation nano-CT to brain tissues of human [1] and fruit fly [2]. Our study reporting the complete reconstruction of brain network of a fly brain hemisphere appeared in *MIT Technology Review* [3].



←Nanotomography at SPring-8

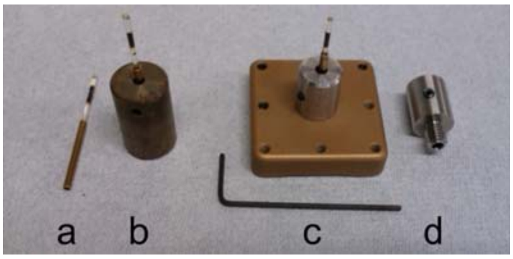
Synchrotron radiation nano-CT was performed at the BL37XU and the BL47XU beamlines of SPring-8, and also at the 32-ID beamline of Advanced Photon Source of Argonne National Laboratory. Recent progress in nano-CT resolved 3D structures at resolutions up to 100 nm. Since the effective pixel sizes are 25-50 nm in most nano-CT experiments, samples are illuminated with high flux x-rays to receive a sufficient number of photons for every pixel of the detector.

Absorption contrast or Zernike phase contrast images produced by 8-keV x-rays were recorded using CMOS-based imaging detectors. Tissue samples were mounted on a precision rotation stage specially built for nano-CT. A total of 900-2400 frames were acquired for one dataset with exposure time of 0.5-1.0 s per frame.



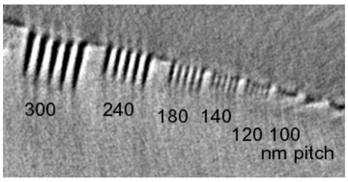
Resin embedding in glass capillaries↑

Biological samples are labile to x-ray irradiation. In order to visualize nanometer-scale structures, samples must be pretreated to make their structures tolerant to the radiation load for over 1000 seconds of the image acquisition. In this study, soft tissue samples were embedded in Petropoxy 154 epoxy resin (Burnham Petrographics, ID, USA), which is available as a petrographic embedding medium. The capillary embedding was performed as shown above [4]. (A) Embedding assembly composed of syringe, 3-way stopcock, and capillary. (B) Fill the capillary with resin. (C) Open stopcock to remove the capillary. (D) Place tissue in the capillary. (E) Insert the capillary and then close stopcock to move the tissue using the syringe. (F) Open stopcock to settle the tissue and cut the capillary. (G) Put the capillary in silicone tubing to cure the resin. (H) Sleeve the capillary with brass tubing.



Capillary samples mounted using dedicated adapters [4]. ↑
(a) Capillary sample of human brain tissue (black) sleeved with brass tubing. (b) Sample mounted on an adapter for SPring-8 beamlines. (c) Sample mounted on an adapter for the 32-ID beamline of Advanced Photon Source. (d) Invar adapter for APS.

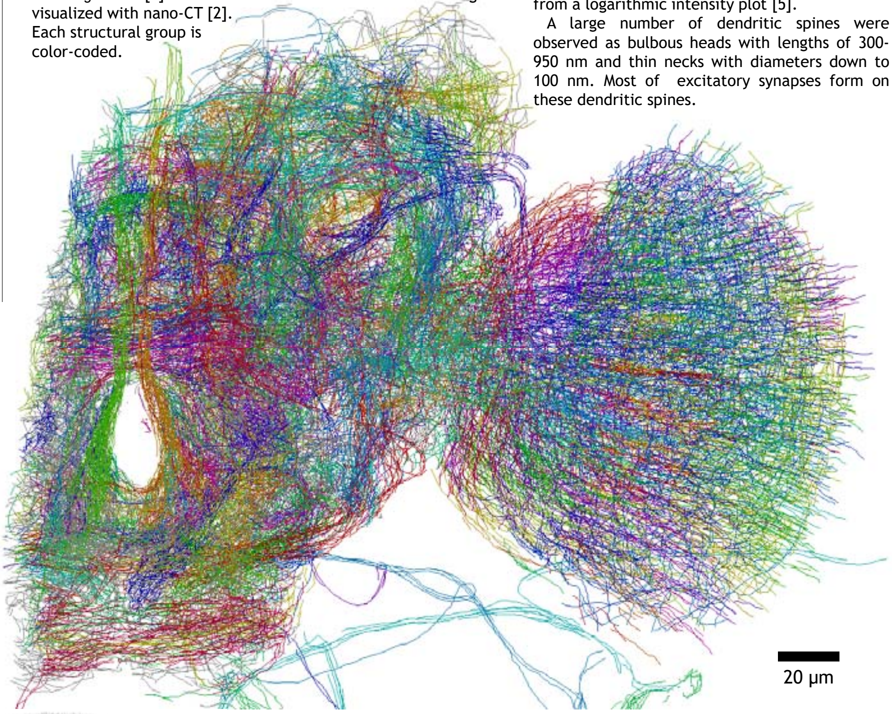
[1] Mizutani, Saiga *et al.* https://arxiv.org/abs/1804.00404
[2] Mizutani, Saiga *et al.* (2013) *J. Struct. Biol.* **184**, 271-279.
[3] First 3-D Map of a Fruit Fly's Brain Network, *MIT Technology Review*, Sep 16 (2016).
[4] Mizutani and Saiga (2018) *Protocol Exchange*, DOI: 10.1038/protex.2018.085
[5] Mizutani, Saiga *et al.* (2016) *J. Microsc.* **261**, 57-66.

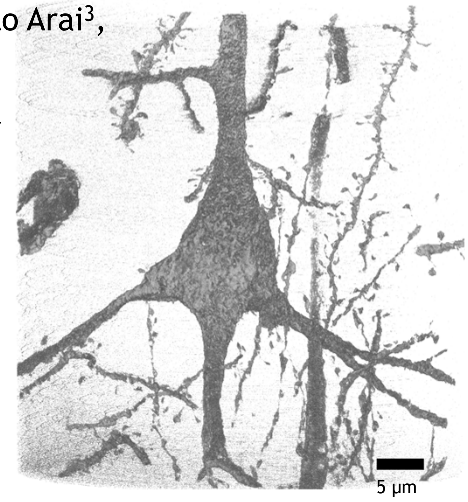


↑A cross section of aluminum test object indicated that patterns up to 120 nm pitches were clearly resolved. Some structures of the 100-nm pitch pattern were also visualized, indicating that the resolution is about 100-120 nm.

Fly brain network ↓

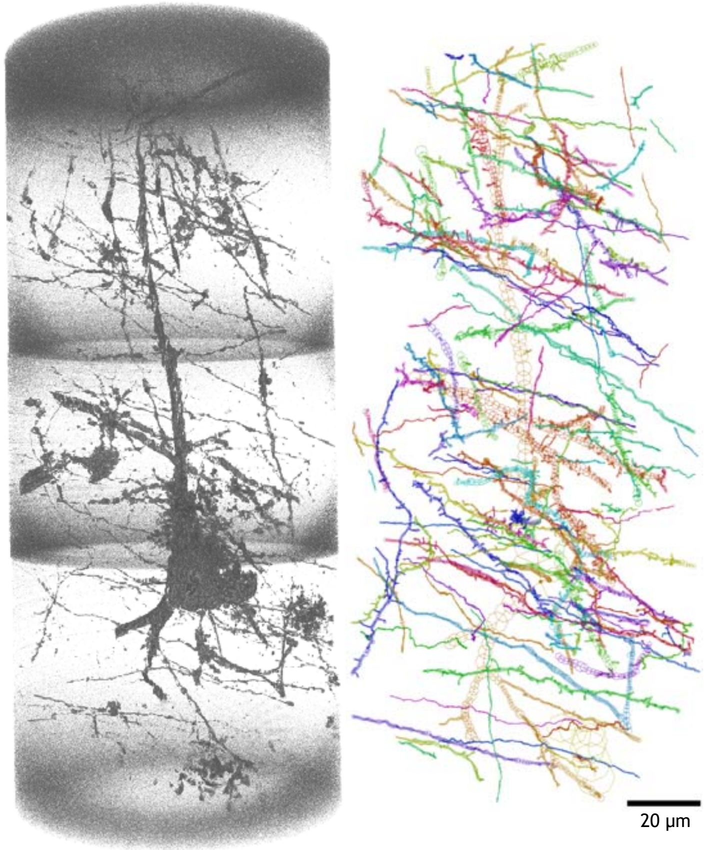
The entire network of brain hemisphere of fruit fly *Drosophila melanogaster* [3] was reconstructed from a 3D image visualized with nano-CT [2].
Each structural group is color-coded.





↑Structure of a human neuron was determined using a nano-CT at BL37XU of SPring-8. The image resolution was estimated to be 118 nm from a logarithmic intensity plot [5].

A large number of dendritic spines were observed as bulbous heads with lengths of 300-950 nm and thin necks with diameters down to 100 nm. Most of excitatory synapses form on these dendritic spines.



←3D Structures of cerebral tissues of schizophrenia and control cases were visualized with nano-CT [1]. Psychiatric symptoms of schizophrenia suggest alteration of cerebral neurons. However, the physical basis of the schizophrenia symptoms has not been delineated at the cellular level. We determined 3D structures of brain tissues of schizophrenia (left) and control cases. Tissue constituents observed in the 3D images were traced to build Cartesian coordinate models of neurons (right). The obtained 55 Cartesian-coordinate models were used for analyzing neuron's geometry, such as curvature and torsion of neurites.

The obtained results indicated that the neurite curvature is significantly different between schizophrenia and control cases. The mean curvature of distal neurites of the schizophrenia cases was approximately 1.5 times higher than that of the controls. A schizophrenia case carrying a frame shift mutation in the GLO1 gene showed the highest neurite curvature, suggesting that oxidative stress due to the GLO1 mutation caused the structural alteration of neurites.

The differences in the neurite curvature result in differences in the spatial trajectory and hence alter neuronal circuits. The tissues analyzed in this study were taken from the anterior cingulate cortex. It has been reported that the anterior cingulate cortex has emotional and cognitive functions. We suggest that the structural alteration of neurons observed in the schizophrenia cases should reflect psychiatric symptoms of schizophrenia.