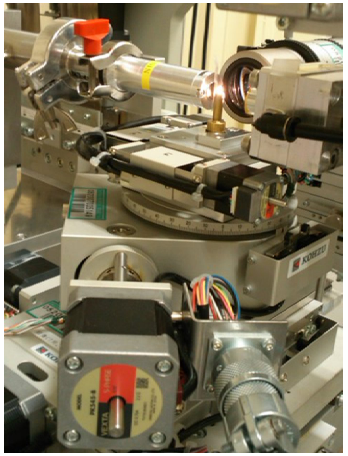
X-ray Tomographic Microscopy of Drosophila Brain Network and Skeletonized Model Building in the Three-Dimensional Image

Ryuta Mizutani*[1], Rino Saiga[1], Kentaro Uesugi[2], Akihisa Takeuchi[2], Yasuko Terada[2] and Yoshio Suzuki[2]
[1] Department of Applied Biochemistry, Tokai University, Japan.
[2] Japan Synchrotron Radiation Research Institute (JASRI/SPring-8), Japan.
*ryuta@tokai-u.jp; http://www.el.u-tokai.ac.jp/ryuta/

Analysis of brain network

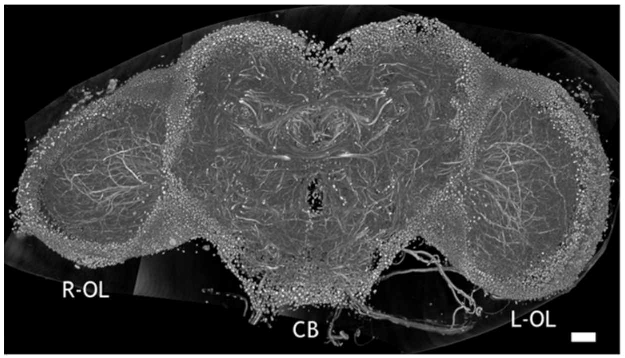
The brain consists of a large number of neurons that make up a 3D network. The first step to understanding brain functions is to analyze the structure of this network. Although 3D structures of brain tissues have been reported, their structures are difficult to comprehend. This is because of a lack of quantitative descriptions of the 3D network, which should be represented with 3D Cartesian coordinates, rather than a 3D distribution of intensities. Here, we report on x-ray tomographic microscopy of the brain network of the fruit fly *Drosophila melanogaster* and its analysis by skeletonized-model building [1].



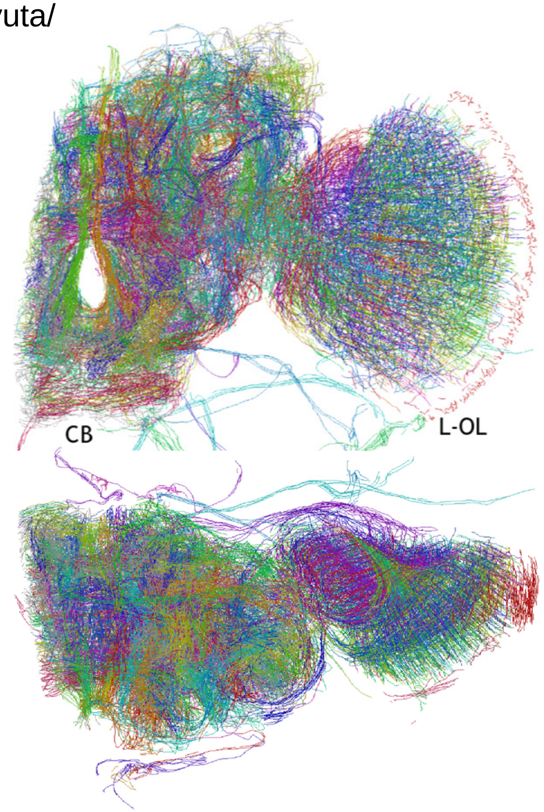
←X-ray microtomography at SPring-8

Fruit fly brains, called as cephalic ganglion, were dissected from a wild-type Canton-S adult flies and stained with aurate by reduced-silver impregnation, as described previously [2].

X-ray tomographic microscopy equipped with Fresnel zone plate optics was performed at the BL37XU and BL47XU beamlines of the SPring-8 synchrotron radiation facility. Transmission images produced by 8-keV x-rays were recorded using a CMOS-based imaging detector. The spatial resolution was estimated to be 160-200 nm (BL37XU) or 600-800 nm (BL47XU) by using micro-fabricated test objects [5].



3D structure of *Drosophila* brain. The anterior half is cut away to illustrate inner structures. Dorsal is to the top. X-ray attenuation coefficients were rendered from 15 cm^{-1} (black) to 100 cm^{-1} (white). Scale bar: 20 µm at the cutaway section.



↑ The entire network of the left hemisphere of the fly brain

In order to analyze the brain structure, its 3D network should be further delineated in terms of Cartesian coordinates by building a skeletonized model. The model was built by using a method like those used in crystallographic studies of macromolecular structures [1,3,4].

The upper panel is a frontal view of the skeletonized model and the lower panel a dorsal view. The left optic lobe (L-OL) and central brain (CB) are labeled. Neuronal process groups are color-coded.

The optic lobe responsible for visual information processing is composed of repeated structures. The central brain, involved in integrative functions, exhibits rather complicated structures.

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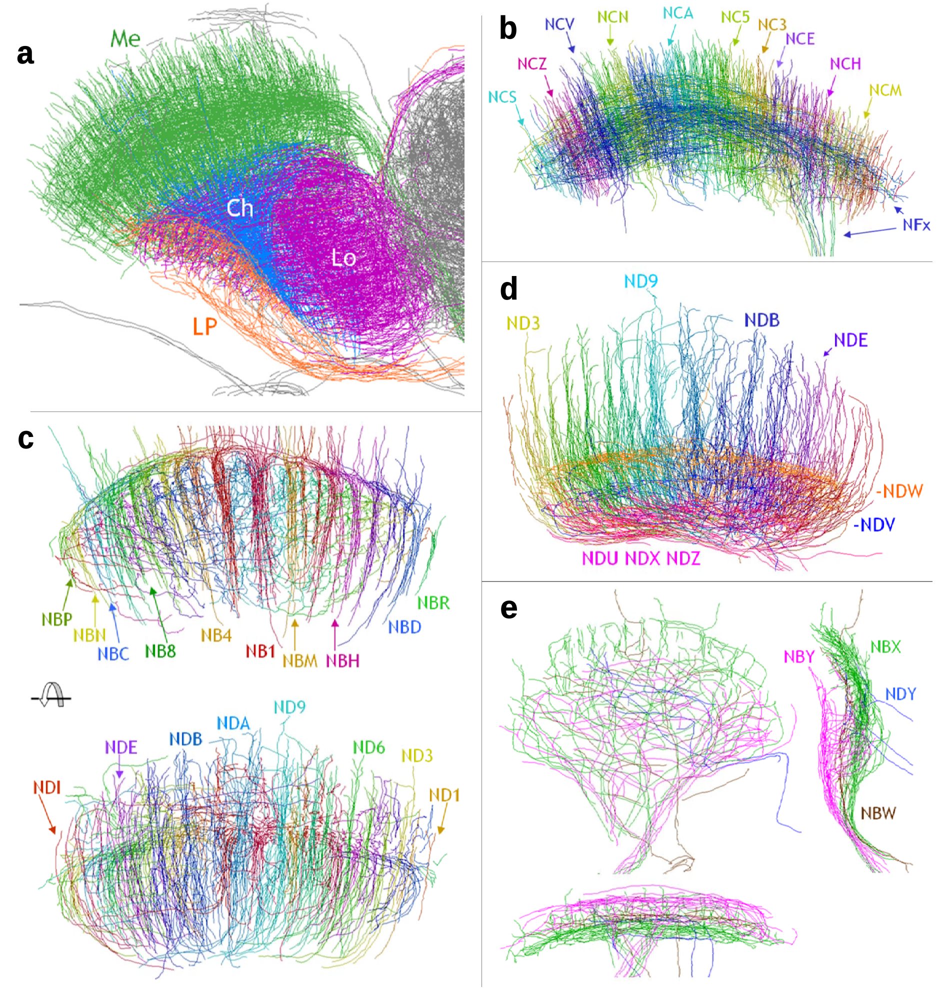
[3] Mizutani *et al*. (2010) *Cerebral Cortex* **20**, 1739-1748.
[4] Mizutani *et al*. (2011) *AIP Conf. Proc*. **1365**, 403-406.
[5] Mizutani & Suzuki (2012) *Micron* **43**, 104-115.



Network structure of the optic lobe ↑

The neuronal process models were classified into groups on the basis of their 3D structures. Anatomical segments can be extracted from the model by specifying neuronal processes in a group-by-group manner. (a) Optic lobe is composed of medulla (Me, green), second optic chiasma (Ch, blue), lobula (Lo, purple), and lobula plate (LP, orange). (b) The medulla is composed of neuronal process groups nearly perpendicular to the outer surface of the brain tissue (NCx) and groups parallel to the brain surface (NFx). (c) The second optic chiasma is structurally divisible into 25–27 segments. In spans of these segments, neuronal processes of the lobula (NDx) are interlaced. (d) The lobula is composed of those interlacing neuronal processes and a cage structure. The cage structure can be classified into distal (NDW), intermediate (NDV) and proximal (NDU/NDX/NDZ) layers.
(e) The structure of the lobula plate. A posterior face of the lobula plate (NBY, magenta) corresponds to vertical motion sensitive neurons. An anterior face (NBX, green) corresponds to horizontal motion sensitive neurons.