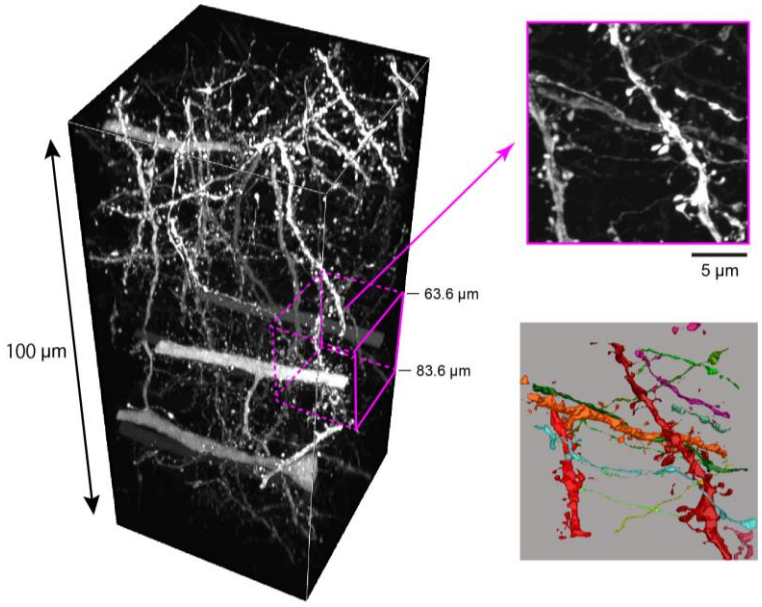
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

Improved tissue clearing solution for super-resolution imaging

April 7, 2016–As biologists seek to gain further insights on subcellular structures deeply embedded in various tissues, high resolution fluorescence microscopy using various fluorescent markers has emerged as a powerful tool to 'see' inside tissues in three-dimension (3D). High resolution imaging remains challenging, however, as it is sensitive to light scattering and spherical aberrations, which in turn creates blurred or inaccurate images. To facilitate 3D imaging, especially of thicker tissue samples, several protocols for turning tissues transparent in a process called optical clearing, have been developed to reduce light scattering and spherical aberrations. While some optical clearing agents achieve very high tissue transparency, many clearing agents include harsh chemicals which can cause damage or swelling of tissues resulting in morphological changes to more delicate structures. Thus, these clearing agents are unsuited for visualizing subcellular features at high-resolution.

Now, in a new work by Foreign Postdoctoral Researcher Meng-Tsen Ke in the Laboratory for Sensory Circuit Formation (Takeshi Imai, Team Leader) and other collaborators, they report the development of a tissue-clearing solution SeeDB2 that can be used in combination with different types of high-resolution fluorescence microscopes, allowing them to analyze tiny fine structures lodged deep within thick tissue samples at super-resolutions. Published in *Cell Reports*, their study also demonstrates the applications of their tissue clearing solution for large-scale detailed 3D imaging of neuronal circuits in the mouse and fruit fly brain at synaptic-scale resolutions.



Reconstructed image of mouse cerebral cortex cleared with SeeDB2 using super-resolution microscopy (Airyscan). Fine structures of the dendritic spines and synapses can be clearly seen.

The Laboratory for Sensory Circuit Formation previously developed SeeDB (see deep brain), a non-hazardous, fructose-based clearing agent (CDB news: Jul. 2, 2013) that can rapidly clear tissues within three days with minimal damage to tissue structures simply by soaking the tissue. Despite the well preserved state of structures in samples treated with SeeDB, obtaining high resolution images of these embedded structures have been hampered by the resolution limit of the objective lenses.

To increase resolution power of fluorescence microscopy, immersion oil, which has a high refractive index (RI), is normally placed between the glass coverslip and objective lens. RIs of clearing agents, including SeeDB, are lower than that of the coverslip and immersion oil, thus when the lens focus shifts deeper in a tissue, the light passing through tissue can no longer converge resulting in spherical aberrations. Spherical aberrations can be minimized when RI of cleared tissues matches that of immersion oil.

The team searched for aqueous solutions with high RIs and came across iohexol, an iodine compound

used as a contrasting medium for X-ray imaging. After testing various formulas, they found that the combination of iohexol, small amounts of saponin (weak detergent) and Tris-EDTA buffer improved its permeation into tissues as well as the transparency of tissues to match the RI of immersion oil and the glass coverslip. This improved formula was named SeeDB2, and tissues could easily be cleared within two days, in some cases as short as a few hours, depending on the thickness of the sample by simple soaking. Like its predecessor, SeeDB2 also minimized tissue swelling during the clearing process, and could stably maintain bright fluorescence signals emitted by several different fluorescent proteins and fluorophores used for high-resolution fluorescence microscopy.

The performance of SeeDB2 was tested using different biological samples in combination with several commercial super-resolution microscopes. When imaging SeeDB2-treated mouse oocytes, which are relatively larger than the average cell, they were able to see individual microtubule strands in fine detail, where previously it had been difficult to do so. In mouse brain tissues cleared with SeeDB2, they were able to visualize neurons located at depths approximately 10 times deeper than previously possible as well as resolve fine neuronal features including spines on the dendritic processes which have diameters less than 1µm.

The team also demonstrated applications of SeeDB2 and super-resolution microscopy for imaging neuronal circuity in brain tissues of mouse. They focused on the neuronal synapses, structures where electrochemical signals between neurons are exchanged, and which due to their small size (< 1µm) have been difficult to observe directly. Many cognitive disorders have been linked to abnormalities of neuronal synapses, thus, understanding synaptic structure will also add to our comprehension of how these disorders arise. They compared the dendrites of normal neurons with those in which an essential subunit of a glutamate receptor NMDAR was knocked out. In normal neurons, dendritic spines displayed different morphologies, some with pointed spines and others that were mushroom-shaped. Whereas excitatory synapses are known to localize at tips of dendritic spines, examination of inhibitory synapses clearly revealed they localize to shaft of dendritic spines as well as the tips. In knockout neurons, while the spine density was comparable to normal neurons, the team observed that sizes of some spines were larger than those of normal neurons and that inhibitory synapses were densely localized at tips of spines. They also successfully imaged the neural circuitry in brain of *Drosophila*, revealing unique synaptic structures in 3D that could not be distinguished before.

“SeeDB2 is a simple and highly versatile clearing agent, particularly useful for imaging fine structures in tissues at high resolutions. The other clearing agents each have their own advantages, therefore scientists should choose the one that best suits their purpose," says Imai. “Electron microscopy has been the only means available until now to study neural synaptic structures, a time-consuming and laborious method. Combining the use of SeeDB2 with super-resolution microscopy will facilitate the study of connectomics of neural networks by revealing a manifold of detailed information on these delicate subcellular structures at synaptic level.”

CDB News: July 2, 2013
http://www.cdb.riken.jp/eng/04_news/articles/13/1300702_seedb.html