Breaking records in neurological microradiology

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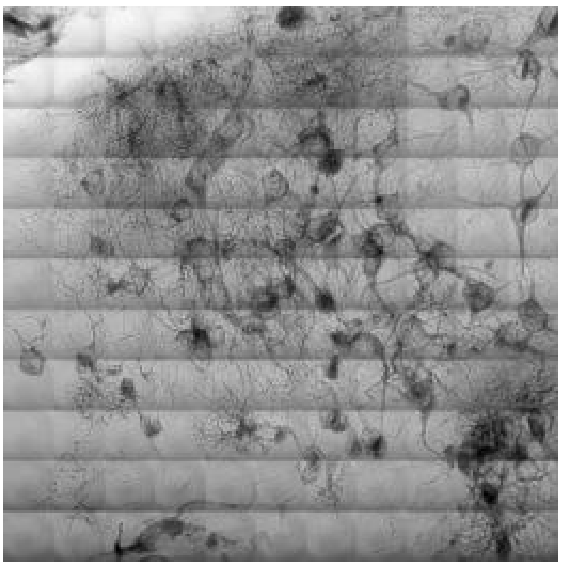

A dense neuron cluster including details of cells in deeper tissue layers.

(Phys.org) -- As neuroscientists probe ever deeper into the mysteries of the brain and nervous system, they need ever sharper vision.

A group of researchers has developed some exciting new techniques for imaging neuronal and synaptic networks using the hard synchrotron x-rays provided by the U.S. Department of Energy Office of Science's Advanced Photon Source (APS).

These techniques provide images with unprecedented detail and resolution, and open the door to three-dimensional tomographic reconstructions, a vital tool for studying the complex tree-like branching nature of neuronal networks.

Understanding intricate neuronal and synaptic networks, particularly in more complex mammalian brains, requires high-resolution mapping of large volumes of tissue, preferably in three dimensions in order to capture all the subtle structural details.

"Mapping neuron networks has been providing a very significant understanding of how the brain works," said Yeukwang Hwu of Academia Sinica in Taipei, Taiwan, lead author of the paper on this new study, which was published in the *Journal of Physics D: Applied Physics*.

While such work has been quite successful in the study of small organisms such as c. elegans and drosophila, the available optical and electron microscopy techniques are not quite up to all of the special challenges posed by neuroradiology, even though they are invaluable for startlingly clear imaging of other types of tissue.

Hwu explains that "methods mostly based on fluorescence and optical microscopy face the problem of not being able to examine larger mammalian brains. Our method takes advantage of the high penetration of hard x-rays and new developments in nanoresolution."

Working at the X-ray Science Division 32-ID x-ray beamline of the APS at Argonne National Laboratory, Hwu and his collaborators from Brookhaven National Laboratory, EPFL in Switzerland, Pohang University of Korea, and National Cheng Kung University in Taiwan achieved significant breakthroughs in neurological microradiology, including a Rayleigh contrast resolution of about 16.5 nm at 8 keV — a world record for resolution with hard x-rays. They examined sections of mouse cerebellum to demonstrate their approach.

The researchers pushed the neuroradiology envelope by using new nanofabrication techniques to create Fresnel zone plates that also acted as magnifying lenses. Their zone plate design achieved a delicate compromise between the narrow structure needed for high resolution and the thickness required to focus the hard x-rays.

This proved to be the key to coupling the penetrating power of x-rays with the extreme resolution required to visualize extremely fine subcellular details.

"Nanoscale x-ray microscopy is still only available with synchrotron x-rays," Hwu said. "Our new level of resolution performance was not possible with the previous state-of-the-art x-ray optics. The high brightness of the x-rays from the APS is another key factor which allows us to pursue very-high-resolution imaging of the fine details of neuron networks."

The team also developed a new staining technique specifically tailored to microradiology. Staining is essential for proper visualization of neurological structures.

But the long-established techniques used for optical microscopy are not workable in microradiology, where thicker specimen samples are the rule, meaning that fluorophores in the staining material can't provide sufficient x-ray enhancement.

After an extensive search for a suitable procedure, the experimenters finally settled on a modification of the mercury and silver-based Golgi-Cox protocol generally used for staining neurons.

By simply by extending the incubation period to a month or longer, they found that the stain was able to perfuse throughout the entire depth of the specimen, allowing visualization of cells in the deepest tissue layers.

These two new techniques not only provided images of unprecedented detail and resolution, but also allowed the generation of three-dimensional tomographic reconstructions, which are a vital tool for studying the complex tree-like branching nature of neuronal networks.

The researchers are already looking ahead to further refining these methods and developing newer techniques of ever greater sophistication.

Hwu explains that the team intends to "fabricate better [higher resolution and efficiency] zone plate optics and develop more stable mechanics. With our collaborations in microbiology and medical research, we will continue the challenging work in neuron imaging to image as many neuron cells as possible to study their relation to neuronal diseases."

Hwu expects the Advanced Photon Source to continue to play a major role in the work: "This high-performance facility provided the best tool for work with hard x-rays, which enabled us to advance the technology and make it available to the scientific community."

More information: H.R. Wu, S.T. Chen, Y.S. Chu, R. Conley, N. Bouet, C.C. Chien, H.H. Chen, C.H. Lin, H.T. Tung1, Y.S. Chen, G. Margaritondo, J.H. Je, and Y Hwu, "Nanoresolution radiology of neurons," *J. Phys. D* 45, 242001 (2012). DOI:10.1088/0022-3727/45/24/242001

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