New microscope records firing of thousands of individual neurons in 3-D

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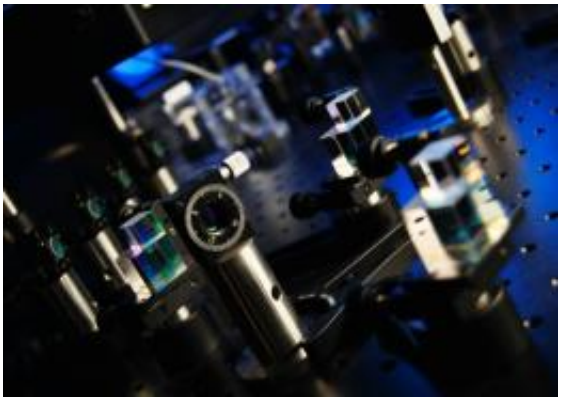

STEM microscope designed at UCLA . Photo credit: UCLA

(PhysOrg.com) -- Some disorders of the brain are obvious -- the massive death of brain cells after a stroke, the explosion in the growth of cells that marks a tumor. Other disorders, such as autism, schizophrenia and mental retardation show no physical signs of damage and are believed to be caused by problems in how brain cells communicate with one another.

To understand the root of the problem of these latter diseases, visualizing brain activity is key. But even the best imaging devices available -- fMRIs and PET scans -- can only give a "coarse" picture of brain activity.

UCLA neuroscientists have now collaborated with physicists to develop a non-invasive, ultra–high-speed microscope that can record in real time the firing of thousands of individual neurons in the brain as they communicate, or miscommunicate, with each other.

"In our view, this is the world's fastest two-photon excitation microscope for three-dimensional imaging in vivo," said UCLA physics professor Katsushi Arisaka, who designed the new optical imaging system with UCLA assistant professor of neurology and neurobiology Dr. Carlos Portera-Cailliau and colleagues.

Their research appears in the Jan. 9 edition of the journal *Nature Methods*.

Because neuropsychiatric diseases like autism and mental retardation often display no physical brain damage, it's thought they are caused by conductivity problems — neurons not firing properly. Normal cells have patterns of electrical activity, said Portera-Cailliau, but abnormal cell activity as a whole doesn't generate relevant information the brain can use.

"One of the greatest challenges for neuroscience in the 21st century is to understand how the billions of neurons that form the brain communicate with one another to produce complex behaviors," he said. "The ultimate benefit from this type of research will come from deciphering how dysfunctional patterns of activity among neurons lead to devastating symptoms in a variety of neuropsychiatric disorders."

For the last few years, Portera-Cailliau has been using calcium imaging, a technique that uses fluorescent dyes that are taken up by neurons. When the cells fire, they "blink like lights in a Christmas tree," he said. "Our role now is to decipher the code that neurons use, which is buried in those blinking light patterns."

But that technique had its limitations, according to Portera-Cailliau.

"The signal of the calcium-based fluorescent dye we used faded as we imaged deeper into the cortex. We couldn't image all the cells," he said.

Another problem was speed. Portera-Cailliau and his colleagues were concerned they were missing information because they couldn't image a large enough portion of the brain fast enough to measure the group-firing of individual neurons. That was the driving impulse behind the collaboration with Arisaka and one of his graduate students, Adrian Cheng, to find a better way to record neuronal activity faster.

The imaging technology they developed is called multifocal two-photon microscopy with spatio-temporal excitation-emission multiplexing — STEM for short. The researchers modified two-photon laser-scanning microscopes to image fluorescent calcium dyes inside the neurons, and came up with a way to split the main laser beam into four smaller beamlets. This allowed them to record four times as many brain cells as the earlier version, or four times faster. In addition, they used a different beam to record neurons at different depths inside the brain, giving a 3-D effect, which had never been done previously.

"Most video cameras are designed to capture an image at 30 pictures per second. What we did was speed that up by 10 times to roughly 250 pictures per second," Arisaka said. "And we are working on making it even faster."

The result, he said, "is a high-resolution three-dimensional video of neuronal circuit activity in a living animal."

The use of calcium imaging in research is already providing dividends. Portera-Cailliau studies Fragile X syndrome, a form of autism. By comparing the cortex of a normal mouse with a Fragile X mutant mouse,

his group has discerned the misfiring that occurs in the Fragile X brain.

Other authors of this study included co-first authors Cheng and J. Tiago Goncalves, and Peyman Golshani, all of UCLA. Funding for the research was provided by the National Institutes of Health. The authors report no conflicts of interest.

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