Enhanced 3-D imaging poised to advance treatments for brain diseases

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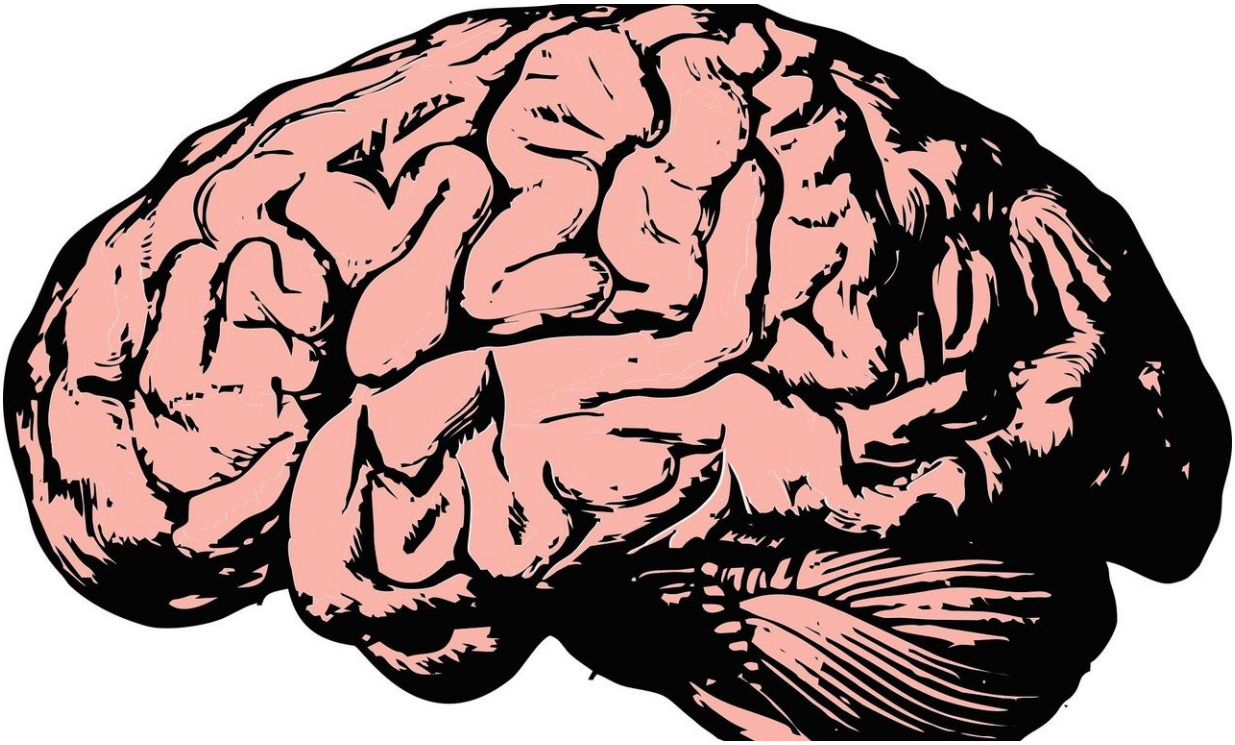

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Researchers have developed a combination of commercially available hardware and open-source software, named PySight, which improves rapid 2-D and 3-D imaging of the brain and other tissues. By seamlessly enabling integration of the fastest 3-D imaging solution available today, the advance in microscopy could help scientists to better understand

brain dynamics and discover new treatments for health problems such as stroke, epilepsy and dementia.

In *Optica*, The Optical Society's journal for high impact research, the researchers describe PySight, which serves as an add-on for laser scanning microscopes. Geared with this novel combination of software and hardware, they improved the quality of 2-D and 3-D imaging of neuronal activity in the living brain.

Because it can image deep into tissue, a laser-based imaging technique known as multiphoton microscopy is often used to study the rapid activity patterns of neurons, blood vessels and other cells at high resolution over time. This microscopy method uses laser pulses to excite fluorescent probes, eliciting the emission of photons, some of which are detected and used to form 2-D and 3-D images.

Trying to capture the full breadth of neuronal activity with multiphoton microscopy forces scientists to image faster. As a result, fewer and fewer photons become available to form images, much like taking a photo with shorter and shorter exposure times. The challenge then becomes how to get meaningful images under these dim conditions.

"To tackle this challenge, microscopists have used a detector-readout method called photon counting," said research team leader Pablo Blinder from Tel Aviv University in Israel. "However, because its implementation required extensive electronics knowledge and custom components, photon counting has never been widely adopted. In addition, commercially available photon counting solutions were ill-suited to perform very fast imaging such as required for 3-D imaging. PySight's easy installation procedure and its integration with state-of-the-art hardware eliminate such concerns."

In addition to advancing neural imaging research, PySight's improved

sensitivity could facilitate rapid intraoperative identification of malignant cells in human patients using multiphoton microscopy. PySight's novel approach for reconstructing 3-D scenes could also improve performance of light detection and ranging, or LIDAR. This could help lower the costs of self-driving cars that use LIDAR to map their surroundings.

Detecting single photons in 3-D

PySight provides high spatiotemporal resolution while producing a data stream that scales with the number of detected photons, not the volume or area being imaged. "Conventional data acquisition hardware stores the brightness of every pixel or 3-D voxel even when it is zero as no photons were detected in that particular location," Blinder explained. "PySight, however, only stores the precise detection time of each photon. If no photons were detected, nothing gets written to disk. This allows researchers to conduct rapid imaging of large volumes over long sessions, without compromising spatial or temporal resolution."

To reconstruct a multidimensional image, knowing when each photon hits the detector isn't enough. It's necessary to also know where it originated in the brain. "This is even trickier if you want to simplify the system and avoid synchronizing the different scanning elements," said Blinder. "To accomplish this, our software reads a list of photon arrival times along with timing signals from the scanning elements, determines the origin of each photon within the sample and generates the corresponding 3-D movies."

The photon arrival times are generated by a device known as a multiple-event time digitizer, or multiscaler, which records the times with a precision of 100 picoseconds. Another key component was an off-the-shelf resonant axial scanning lens that changes the focal plane hundreds of thousands of times per second. This lens was used to rapidly scan the

laser beam across different depths within the brain and allowed the team to reconstruct continuous 3-D images.

Easier, cost-effective, continuous 3-D imaging

"The multiscaler we used hasn't been applied to neuroimaging because the output isn't easy to interpret, and using a resonant axial scanning lens for bioimaging has required custom-made scanning synchronization hardware or proprietary code to obtain the 3-D data," said Blinder. "PySight turns the output from both components into a 3-D movie effortlessly. As Pysight is free and open-source software, it should greatly aid labs previously deterred by the high technical barrier that accompanied continuous 3-D imaging." Further, having a generic application interface, PySight could also be used to interpret similar photon detection times from other suitable hardware devices.

To test whether PySight was truly plug and play, the researchers walked with their multiscaler to another imaging lab on the Tel Aviv University campus. They were able to simply plug in the device into the existing multiphoton microscope, download the PySight software and start recording single-trail odor responses in fruit flies genetically modified to express voltage indicators. This fast probe for neuronal activity detects the finest aspects of neuronal activity yet it is considered too dim to be used without photon counting with this type of microscopy. PySight capabilities pave the road for and easy implementation of multiphoton voltage imaging in almost any laboratory.

In addition to continuing to improve the PySight software, the researchers would like to add support for other microscopy imaging methods such as fluorescence lifetime imaging, which relies on the timing of each photon relative to its originating laser pulse. Because the software is open source and provides direct access to photon arrival times, it enables other scientists to add new features and meet their

specific needs.

More information: H. Har-Gal, L. Golgher, S. Israel, D. Kain, O. Cheshnovsky, M. Parnas, P. Blinder, "PySight: plug and play photon counting for fast continuous volumetric intravital microscopy," *Optica*, 5, 9, 1104-1112 (2018). DOI: 10.1364/OPTICA.5.001104