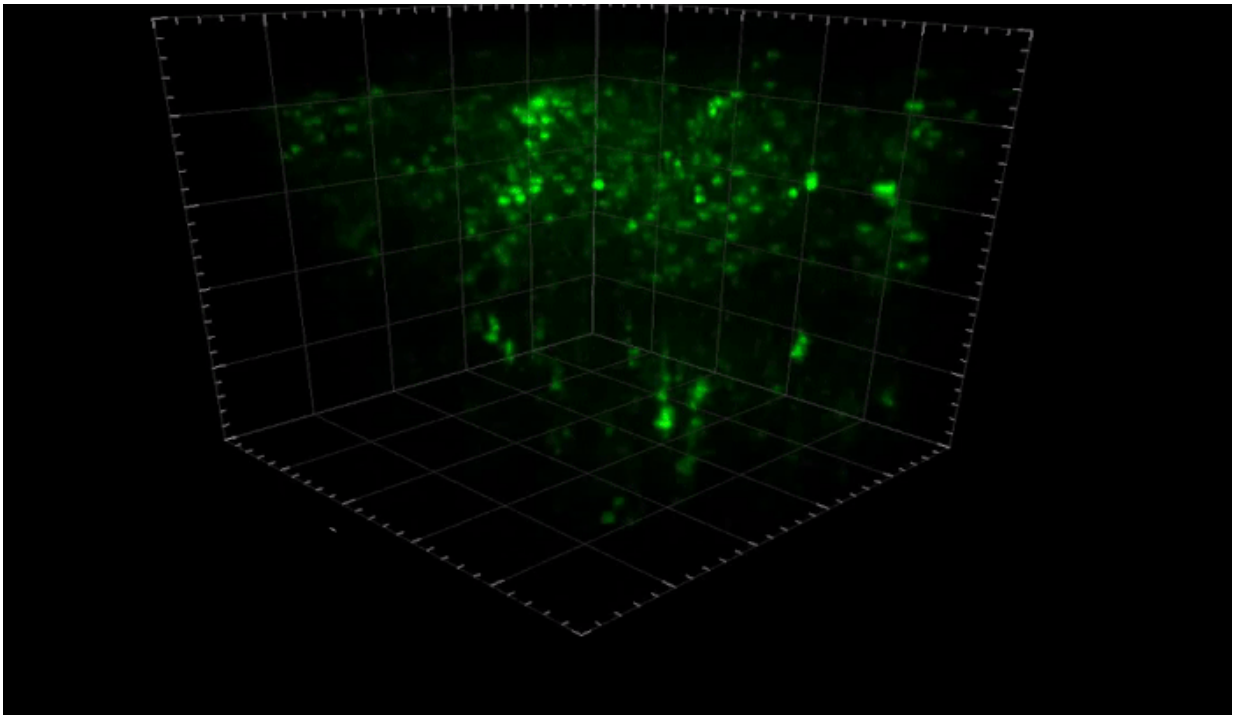


Researchers watch in 3-D as neurons talk to each other in a living mouse brain

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Neurons within a three-dimensional section of mouse brain, in a movement-planning region, light up as they signal to one another. The neurons were genetically altered to fluoresce upon taking in calcium ions, which happens when neurons are active. Credit: Laboratory of Neurotechnology and Biophysics at The Rockefeller University/*Nature Methods*

No single neuron produces a thought or a behavior; anything the brain accomplishes is a vast collaborative effort between cells. When at work,

neurons talk rapidly to one another, forming networks as they communicate. Researchers led by Rockefeller University's Alipash Vaziri are developing technology that would make it possible to record brain activity as it plays out across these networks.

In research published October 31 in *Nature Methods*, they recorded the activity of thousands of [neurons](#) layered within three-dimensional sections of [brain](#) as they signaled to one another in a living mouse.

"The ultimate goal of our work is to investigate how large numbers of interconnected neurons throughout the brain interact in real time and how their dynamics lead to behavior," says Vaziri, an associate professor and head of Laboratory of Neurotechnology and Biophysics. "By developing a new method based on 'light sculpting' and using it to capture the activity of the majority of the neurons within a large portion of the cortex, a layered brain structure involved amongst others in higher brain function, we have taken a significant step in this direction."

This type of recording presents a considerable technical challenge because it requires tools capable of capturing short-lived events within individual cells, all while observing large volumes of brain tissue.

Vaziri, who joined Rockefeller last year, began working toward this goal about six years ago while at the Research Institute of Molecular Pathology in Vienna. His group first succeeded in developing a light-microscope based approach to observing the activity within a whole 302-neuron roundworm brain, before moving on to the 100,000-neuron organ of a larval zebrafish. Their next target, the mouse brain, is more challenging for two reasons: Not only is it more complex, with about 70 million neurons, but the rodent brain is also opaque, unlike the more transparent worm and larval fish brains.

To make the activity of neurons visible, they had to be altered. The

researchers engineered the mice so their neurons could emit fluorescent light when they signal to one another. The stronger the signal, the brighter the cells shine.

The microscopy system they developed had to meet competing demands: It needed to generate a spherically shaped spot, slightly smaller than the neurons and capable of efficiently exciting fluorescence from them. Meanwhile, it also had to move quickly enough to scan the activity of thousands of these cells in three dimensions as they fire in [real time](#).

The team accomplished this using a technique called "light sculpting," in which short pulses of laser light, each lasting only a quadrillionth of a second, are dispersed into their colored components. These are then brought back together to generate the "sculpted" excitation sphere.

This sphere is scanned to illuminate the neurons within a plane, then refocused on another layer of neurons above or below, allowing neural signals to be recorded in three dimensions. (This was done while the mouse's head was immobilized, but its legs were free to run on a customized treadmill.)

In this way, Vaziri and his colleagues recorded the activity within one-eighth of a cubic millimeter of the cortex, of the animal's brain, a volume that represents the majority of a unit known as a cortical column. By simultaneously capturing and analyzing the dynamic activity of the neurons within a cortical column researchers think they might be able to understand brain computation as a whole. In this case, the section of cortex studied is responsible for planning movement.

The researchers are currently working to capture the activity of an entire such unit.

"Progress in neuroscience, and many other areas of biology, is limited by

the available tools," Vaziri says. "By developing increasingly faster, higher-resolution imaging techniques, we hope to be able to push the study of the brain into new frontiers."

More information: Fast volumetric calcium imaging across multiple cortical layers using sculpted light, *Nature Methods*, [DOI: 10.1038/nmeth.4040](https://doi.org/10.1038/nmeth.4040)

Provided by Rockefeller University

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