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A Bright Idea for a New Kind of Microscope

A new laser microscopy imaging technique that uses the principles underlying holography is making it possible for researchers to greatly speed up imaging and to optically manipulate living cells in any pattern in space or time, according to new research published by a team of Howard Hughes Medical Institute (HHMI) scientists.

Biologists discovered the utility of lasers more than 20 years ago, and have since used them to shine concentrated beams of light at cells and parts of cells under a microscope. Ultrafast lasers have enabled a technique known as two-photon excitation, which allows scientists to image and optically manipulate living samples with unprecedented detail. But since lasers illuminate such small focal points, it can take seconds to scan a whole field of cells to build a comprehensive image. Indeed, researchers using two-photon lasers have difficulty detecting, for example, the signals that race from neuron to neuron in milliseconds.

"This is our keyboard to play the piano with neural circuits."

- Rafael Yuste

In the current study, HHMI investigator Rafael Yuste and his team at Columbia University combined elements from a two-photon laser microscope with a spatial light modulator (SLM), a small device that modifies the phase of light and splits a single laser beam into any pattern of the researchers' choosing. At first, they used the eminent Spanish neurobiologist Santiago Ramón y Cajal for inspiration, and shaped the laser beam into his likeness. They shined this light onto a gel infused with fluorescent dye, and saw a two-photon fluorescence portrait of Ramón y Cajal. Yuste and his research group reported their experiments in the December 2008 issue of *Frontiers in Neural Circuits*.

As a next step, the researchers used their new tool with living biological samples. First, they captured an image of neurons under the microscope. Then they used this picture as a template for the SLM, and generated a new pattern of laser light. They used that light pattern to simultaneously stimulate specific neurons or dendritic spines on those neurons -- much like a musician plays the notes or chords on an instrument. "This is our keyboard to play the piano with neural circuits," Yuste says. In order to attempt such an experiment without an SLM, the researchers would have to play one "note" at a time by pointing a two-photon laser beam at a spine on the first neuron, then the second, and so on.

For more than 20 years, Yuste has been trying to determine how brain circuits are wired, using lasers to both detect and activate neurons. In the experiments reported in *Frontiers in Neural Circuits*, the researchers also tried to see how fast they could follow the electrical activity of specific neurons within a slice of mouse brain. Yuste's group found that by illuminating only the neurons of interest, the SLM allowed them to trace the activity of individual neurons at a rate of one image every 16 milliseconds. Previously, a single two-photon frame would take an entire second.

The new technology can give researchers incredible flexibility with lasers, Yuste says. "In this paper, we do a couple of the things we always dreamt of doing," he says. "For instance, we take a single two-photon laser, split it into several spots, and use those spots to turn on neurons simultaneously or to turn on multiple parts of the same neuron simultaneously."

About three years ago, frustrated with the slow speed of two-photon laser imaging, Yuste's group purchased fixed beam splitters. Such devices, which are commonly used in laser light shows, use diffraction to divide light from a single laser into set of fixed patterns, such as circles or lines. But because the exact location of neurons in the brain is variable, Yuste wanted more flexibility in the patterns of laser light hitting their brain slices. "That's when we decided to try a different type of beam splitter, a computer-programmable beam splitter," he recalls.

With funds from HHMI, Yuste's group bought an SLM. The phase-only SLM is a tool used to manipulate the phase of light waves and create holograms. Holography - a method for recording and reconstructing light waves scattered from an object-- was first proposed as way to improve the resolution of electron microscopy by Hungarian scientist Dennis Gabor in the late 1940s. That idea came to fruition after lasers were developed several decades later.

Biologists were relatively inexperienced with diffractive SLMs, and Yuste's group hadn't heard of others doing such work. Nevertheless, the group unpacked the new SLM, integrated it into the microscope, and figured out how to control it. In a few months, they were able to produce the first functional images with their new tool. During that time, they also made sure the laser-SLM combination would work in perfect harmony -- that is, that the powerful ultrafast laser wouldn't destroy the SLM, and the SLM wouldn't render the ultrafast laser's light useless.

In August 2008, a group of French scientists, working independently from the Columbia/HHMI team, published research on a similar approach. That group used a conventional one-photon laser and an SLM to selectively excite branches of neurons. Their work and that from Yuste's group strengthen the idea that SLMs can reduce the time bottleneck that has limited laser-based microscopy's usefulness in dissecting cell signaling, Yuste says.

Yuste envisions a future in which an SLM is used in every laser microscope. His group is working on a simple prototype of the microscope used in the experiment, as well as a small "pocket scope," consisting of an SLM and objective lens. Many of the microscope's hardware functions can be transferred to the phase-only SLM. A user-friendly interface can hide all of the complex functions, he says.

"This is the beginning of what I think will be the future of microscopy, where a lot of the sophisticated optical manipulations of light will be done with software instead of hardware," Yuste says.