A giant leap in detecting brain activity, thanks to glowing voltage sensors and a “disco ball”

BU Photonics researchers developed a new method and microscope allowing them to see electrical activity between brain cells in exquisite detail

By Kat J. McAlpine

A new microscopy approach — capable of imaging the voltage of brain cells as they process information — uses genetically-engineered proteins and an ultrafast microscope (with an onboard, light reflecting “disco ball”) to measure voltage inside individual neurons and within networks of brain cells, phenomena that happens incredibly fast and has been very difficult to observe until now.

As you read this, electrical activity is ping-ponging between cells in your brain at very high speeds. There are about 86 billion neurons inside the human brain, working together to transmit information that empowers thoughts, movements, perception, behavior, and regulatory processes throughout the human body. That cacophony of activity happens automatically, without us making any conscious effort, as brain cells fire electrical discharges that send signals and information between cells.

“Voltage is the way information is transferred within the brain,” says Michael Economo, a Boston University Photonics Center faculty member and a College of Engineering assistant professor of biomedical engineering.

Detecting voltage changes is at the crux of understanding brain activity and how neurons orchestrate complex behaviors of the body and mind. But historically the methods to image electrical activity within the brain have only allowed scientists to glean its presence indirectly, such as a technique called calcium imaging, which detects fluctuations in calcium that hint at voltage changes inside cells.

That’s finally changing, thanks to a new advance in voltage imaging that’s giving neuroscientists the most detailed look yet at how voltage travels through brain cells. “This advance has really broad implications for neuroscience,” says Economo, one of the principal investigators on the project.

He and his lab team at BU are focused on understanding exactly how mammalian brains work down to the level of single cells. To make advances in voltage imaging, they joined forces with their next-door neighbors at BU, who happened to be the perfect collaborators—a lab group led by Jerome Mertz, who has spent his career inventing new microscopic imaging technologies.

“Our labs are right next to each other, and when I started my group at BU in April 2019, right off the bat we knew we both had interest in voltage imaging,” Economo says.

Together, Economo and Mertz's labs have developed an approach for detecting voltage between brain cells in exquisite detail using new techniques and a huge dose of creativity. Their work builds upon research by a team at the Howard Hughes Medical Institute (HHMI) Janelia Research Campus, who within the last decade have discovered better ways to engineer proteins so that they more strongly "light up"—or fluoresce—when a cell's voltage changes.

"The tool, called Voltron, lets researchers track neuron activity in living animals more precisely and for far longer time periods than was once possible," according to a 2019 press release describing the breakthrough.

"Previously, when scientists were doing voltage imaging in tissues or cells, they could only see one or two cells at a time," says Mertz, a BU Photonics faculty member, College of Engineering professor of biomedical engineering and of electrical and computer engineering, and College of Arts & Sciences professor of physics. "The big issue that presented for someone like Mike [Economo] is that he wants to look at populations of neurons to see how they're talking to one another."

Working in tandem, Economo's team began experimenting with using the voltage sensor Voltron in mouse brains, while Mertz's lab designed an ultrafast microscope and imaging approach quick enough and sophisticated enough to "see" rapid shifts in voltage across many different cells at the same time.

"It's the advances in the voltage sensor technology, more so than advances in microscopy, that had created a bottleneck before," Mertz says. "No one wants to build a microscope to image a sensor that doesn't exist yet. New sensors [like Voltron] lead to new scopes that allow us to use them."

The dream of voltage imaging is becoming a practical reality

"It's incredible to see how far we've come in voltage imaging in just five or six short years," says Tim Weber, a postdoctoral researcher at Massachusetts Institute of Technology who earned his PhD in Mertz's lab, and led development of the new microscope for voltage imaging.

"I was recruited to Mike [Economo]'s lab because he wanted to create a new system that would allow us to use voltage imaging to acquire large amounts of connectivity data in brain tissue," says Vicky Moya, a postdoctoral researcher at BU. "While I was developing a set-up for how to best use the latest Voltron sensors in brain tissue, and how to best prepare those tissues for imaging, Tim was coming up with the best microscopic design to allow us to 'see' voltage."

"The biggest challenges facing voltage imaging have always been imaging speed and lack of signal," Mertz says. "With a stronger signal now possible because of Voltron, that opened the door for us to work on increasing imaging speed."

At the heart of the solution? A disco ball of sorts.

“The system we developed is built upon a confocal laser scanning microscope,” Weber says. This type of microscope, widely used by scientists, comprises mirrors that direct or scan a focused laser beam to hit specific points and depths within a sample, creating a three-dimensional, detailed image. The mirrors, powered by motors, move quickly so that a sample can rapidly be scanned. But those motors don’t move fast enough to capture the fluorescence bursts given off by neurons during voltage imaging.

“We shopped around for the fastest laser-deflection hardware and discovered that instead of using traditional mirrors mounted on motors, you could instead put a polished polygonal mirror” — shaped close to a sphere and covered in 128 facets of mirrors — “on a continuously spinning motor.” This approach, creating a tiny, rapidly moving “disco ball” inside the microscope, allows the team to drastically accelerated the imaging speed. “You can scan 117,000 lines per second instead of the 8,000 lines you can do with a conventional scope,” Weber says.

The HHMI researchers who developed Voltron sent a supply of it to Economo’s lab so that the team could test out the sensor in mice and use it with new microscope Weber designed. To introduce Voltron in to lab mice, Moya injects a virus containing the gene for the voltage-sensing fluorescent protein (aka Voltron) into the brain, triggering the brain cells to produce Voltron. “They gave us the virus [containing the Voltron gene] with the agreement that we would share our experimental data with them as we collected it,” Moya says.

Once the mice’s neurons produced enough Voltron to emit light as they fired, the mice were euthanized and Moya carefully prepped delicate slices of their brain tissue for imaging. “I put the brain tissues into a solution that supports all the electrophysiology of brain cells, including sugars, sodium, and potassium. The solution is also flooded with oxygen. If you keep the tissue in this solution, the cells can survive outside the body for several hours, giving us plenty of time to look at tissue samples under the microscope to see where the Voltron sensor appears.”

To make sure Voltron was working as anticipated, Moya initially needed to perform the painstaking process of comparing Voltron to an old standby for measuring electrical activity in cells: a technique called patching.

“You have to validate a new approach to what the standard was before,” Moya says. “During patching, you use a delicate glass pipette to physically ‘pop open’ and enter a brain cell, measuring the voltage of that one individual brain cell. Using this approach, you can analyze a couple of cells per day. That’s how I did all my research as an undergraduate, and is the way everyone has been measuring voltage reliably.”

Patching countless individual cells to make sure the Voltron sensor was accurately signaling electrical activity, Moya found that Voltron works exceedingly well — and at near lightspeed compared to the time and labor-intensive patching process. “Seeing how well it worked, with such high fidelity and speed, changed my perspective on what I think is possible in

neuroscience. There's no end to the questions you can ask by applying this technique," she says.

A "super cool" moment for voltage imaging

This milestone is a bit of a rocket for Voltron's odds of becoming a practical tool in neuroscience, given the relative speed and ease in which Weber's scope can detect the sensor. "The previous state of the art in voltage imaging required scientists to repeat experiments over and over and over with much slower microscopes, to cancel out "noise" and accurately detect a signal — indicating voltage change — from fluorescing brain cells," Weber says.

"Our scope is based on confocal laser scanning microscopy," Mertz says. "The idea is to collect good light — from the Voltron sensor — while rejecting 'bad' background light."

Once the team saw that they could accurately detect voltage in brain slices using the new system, they quickly began dreaming of an even more ambitious goal: using the system to map out how voltage travels in the brain circuits of living mice, or what scientists refer to as *in vivo.*

"Initially, we wanted to try this out of curiosity — we weren't sure how well it would work," Moya says.

Neuroscientists already have tried-and-true methods for studying the brains of living mice using other types of imaging techniques. This usually involves performing surgery on the mice to replace a part of the skull with fine layers of glass, which allow scientists to peer into the brain while keeping the mouse's brain protected from the external environment. Using this method, Moya began using Weber's new microscope to look for Voltron signals in living mice.

"I was imaging the brains of mice as they were awake and performing spontaneous movements and activities," she says. "Then, intermittently, I would feed them little drops of water to see what happened in the brain as they performed licking and drinking behaviors. I wasn't trying to target any areas of the brain in particular; I was more interested to see how well our approach would work in living animals."

To her surprise and delight, Moya saw brain cells lighting up with Voltron all over the place. "It was super cool!" she says.

"Vicky would send me little snapshots of the data as she was capturing it, and my mind would be blown," Weber says.

"When we realized it would work in imaging living animals, we started wondering how deep can we push this technology?" Moya says.

"The goal is to image brain tissue, but brain tissue is not transparent," Mertz says. When imaging brain activity in living mice, scientists can typically only glean activity happening on the

surface of the brain. “The deeper you want to see inside the brain, the harder it is to image. But that’s one of our goals in developing new microscopy — figuring out to image brain tissue as deeply as possible.”

“The real value for neuroscientists is to be able to image across depths of the brain, rather than just the surface of the brain,” Moya says. To make that possible, the team surgically implanted an incredibly small, mirrored prism into the mouse brain. They also used an extremely thin, single layer of glass to protect the brain. The implanted prism sends light downward, bouncing it off a 45-degree mirrored surface, and illuminates a vertical region of tissue that stretches 750 microns (three-quarters of a millimeter) deep into the brain.

A new frontier for voltage imaging

Combined with their voltage imaging system, the team is now able to capture volumetric data about electrical activity within networks of brain cells. They are currently capable of imaging up to 50 cells at the same time and are working toward imaging even greater numbers of cells simultaneously.

“No one has ever achieved this with voltage imaging,” Moya says. “We’re now using this approach to suss out how cells are connected within neural circuits. And we’re performing molecular analysis to pinpoint exactly which types of cells make up each circuit.”

Today, as a postdoc at MIT, Weber is developing microscopes for rapid imaging of surgical tissue, with the goal of enabling clinicians to immediately look at the cellular and molecular makeup of surgical excisions or biopsies during the process of surgery itself. The work will help surgeons make sure they’ve successfully removed all cancerous cells, for example, while they are resecting a tumor. Working so closely with Moya has given Weber a new perspective on the ingredients that make up powerful imaging systems.

“As a tech and hardware guy, I now have such greater appreciation for the importance of tissue preparation,” he says. “The very best microscope won’t help you if the tissue isn’t prepared perfectly — you can’t engineer your way out of that.”

He is also still involved in the voltage imaging work with Economo and Mertz’s teams. Combining all these new techniques, the researchers hope to establish a totally new platform for understanding the inner workings of neurons.

“I went to a neuroscience conference recently and noticed that a lot of people think negatively about practical applications of voltage imaging in research. ‘You can’t get enough signal,’ they say, or ‘you can only use it right at the surface of the brain.’ In general, people just sounded very pessimistic about where this could go,” Moya says. “So, I pointed those folks in the direction of our preprint publication, which describes the progress we’ve made. This is opening up a whole new field of neuroscience research, and building on the work of so many others, we’re really grateful to be helping put this method out into the world.”