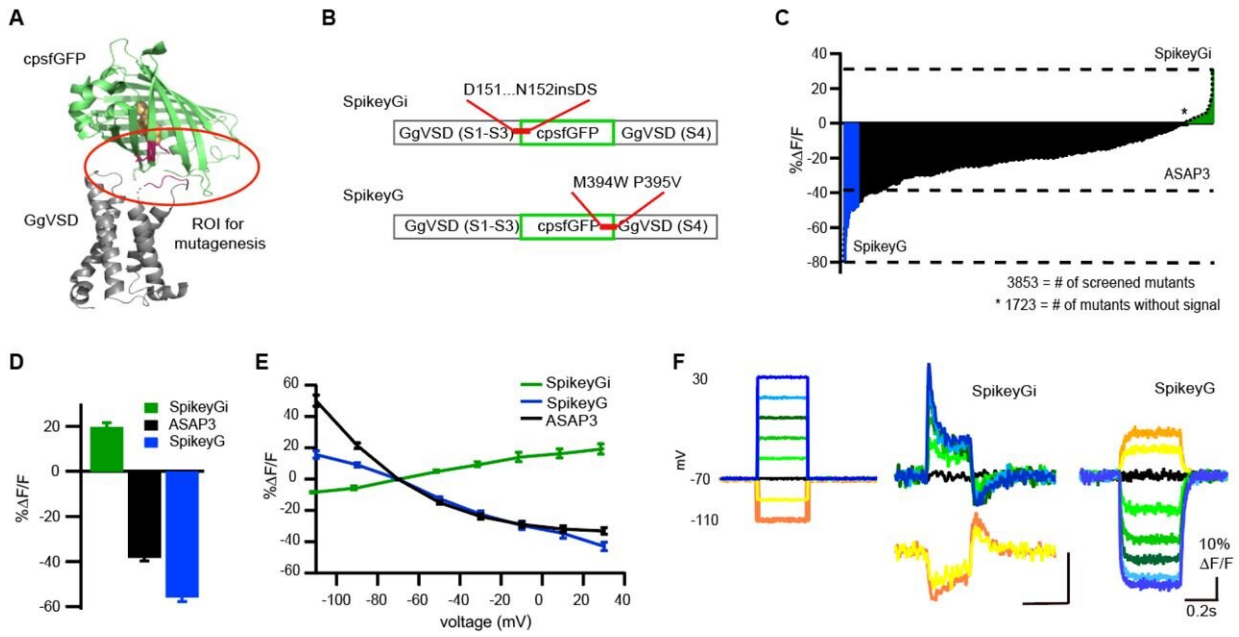
Experts use new microscope, AI algorithm, and voltage indicators to image electrical activity deep in the brain

March 27 2023, by Katherine Gianni



Design and functional characterization of green, positive-going, two-photon-compatible GEVIs. Credit: *Nature Methods* (2023). DOI: 10.1038/s41592-023-01820-3

When studying the brain, researchers are just beginning to use a method known as voltage imaging to track neural activity in the living animal. While this approach is a promising way to better understand neuron firing, behavior, and cognition, there are limitations and risk factors. The

practice requires putting a lot of light into the brain (which can lead to overheating) and only has the capacity to image ten neurons at a time.

New research from Jerry Chen, a Boston University College of Arts & Sciences assistant professor of biology, and collaborators aims to address these challenges. Published today in *Nature Methods*, Chen and co-authors outline how their multidisciplinary approach using a new microscope, artificial intelligence algorithm, and voltage indicators can enhance the imaging process. Taken together, their methods resulted in successful, minimally invasive, and sustained imaging of approximately 100 neurons at a time in mice.

In this Q&A, Chen describes the new research findings, working with his collaborators, and the exciting implications for patients with epilepsy and future brain imaging technologies.

In your own words, please describe this study. What are your main research objectives?

Neuroscience, as a field, is interested in understanding how the brain works. Electrical signals are the primary way in which neurons compute information and communicate with each other. We can use electrodes to record the activity from individual neurons, but this is an invasive procedure that requires inserting electrodes into the brain.

Imaging voltage signals provides a way to non-invasively read out activity across populations of neurons. There has been a lot of effort in the past decade to advance this voltage imaging technology. Our research objective was to help make it practical and scalable for research applications.

Is there a specific challenge this research aims to

address?

Voltage imaging in the living animal means having to operate at the fundamental limits of both physics and biology. We need to express genetically encoded indicators that will change fluorescence in response to neuronal activity and we need microscopes that will allow us to image at very high speeds (at least 1000 frames per second) to measure action potentials (the main unit of information in neurons).

Using our microscopes, we need to put just enough light into the brain to get fluorescence signals out. If we want to image from more and more neurons, we want to put more light into the brain. However, we can't put too much into the brain otherwise we will cause photodamage. So the challenge is walking a tightrope between maximizing the signal and number of neurons we want to record at high-speed and minimizing the chance of causing damage to the brain.

You've described this work as a "multi-disciplinary approach," as you collaborated with co-authors trained in cellular and molecular physiology, biomedical engineering, neurophotonics, and more. Can you elaborate on how these unique disciplines came together to support/influence different areas of the study?

In order to overcome the challenges I described above, there is no one solution that can do this. Instead, you need multiple approaches that will work in combination to overcome these fundamental limits. Specifically, we needed protein engineers that could develop more sensitive voltage indicators that will fluoresce in response to neuronal activity.

As an optical engineer, I developed a new microscope that allows us to increase the number of neurons that we can image at very high speeds. Finally, we needed a computer scientist who could develop new algorithms using artificial intelligence that can extract the voltage signals under noisy conditions in which we are putting very little light into the brain.

Who are the key research collaborators?

Vincent Pieribone at Yale University's John B. Pierce Laboratory developed the new voltage sensors. Lei Tian, an assistant professor within BU's department of electrical and computer engineering developed the new denoising algorithms. Anderson Chen, senior imaging scientist with BU's Neurophotonics Center and manager of the micro and nano imaging core facility, provided guidance in building our SMURF microscope. Ian Davison, associate professor in BU's biology department, helped with testing the voltage sensors in our lab.

Through this study, did you uncover any results that surprised you?

I believe the denoising algorithm (called DeepVID) that Lei Tian developed is a game changer. When you're imaging under low light conditions, the images that you collect can be very noisy. This is called shot noise. This is a fundamental limit in microscopy that prevents us from making reliable measurements using our instruments. The computational methods Lei developed breaks this fundamental limit. I was amazed when I saw how much more easily we could see the voltage signals after applying Lei's denoising algorithm.

What are some of the long-term implications of the research findings? How can the results be applied to

real-world patients or impact the future of brain imaging technologies?

The long-term implication is that we have developed a combined approach that will allow us to scale up voltage imaging. Before, we could only perform imaging with 10 neurons at a time. Our paper demonstrated that 100 neurons is possible. By extending on the principles that we have proved possible in practice, we should be able to image 1000 or more neurons. This will allow us to better understand how information is processed in the brain. It will also allow us to better study diseases in which electrical activity is disturbed like during epilepsy.

What are the next-steps for you and your collaborators?

Our next steps are to both apply our technologies to answer fundamental questions in neuroscience and also explore avenues for further scaling up this imaging towards larger neuronal populations.

More information: Vincent Pieribone, High-speed low-light in vivo two-photon voltage imaging of large neuronal populations, *Nature Methods* (2023). DOI: 10.1038/s41592-023-01820-3.
www.nature.com/articles/s41592-023-01820-3

Provided by Boston University