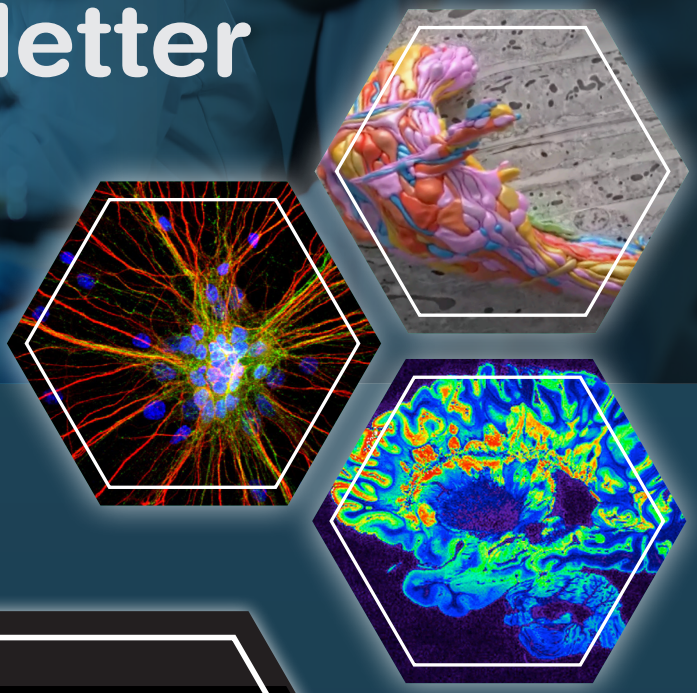
AUGUST 2022

Toolmakers Newsletter



ISSUE 07

In this issue

BRAIN INITIATIVE

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Welcome Back to the Toolmakers Newsletter!

Welcome to the third *Brain Research Through Advancing Innovative Neurotechnologies®* (BRAIN) Initiative Alliance Toolmakers Newsletter of 2022!

In this issue, we are excited to highlight recent developments and inner workings of four more neuroscience programs and tools: NeuroNex: Cornell by Dr. Joe Fetcho, Dr. Chris Xu, and Dr. Chunyan Wu; NeuroNex: Bioluminescence Hub by Dr. Christopher Moore; Open Ephys Graphical User Interface (GUI) by Dr. Josh Siegle; and Michigan µLED Probes by Dr. Euisik Yoon and Dr. Roberto Lopez. Let's learn more details about these projects and some insights from the investigators!

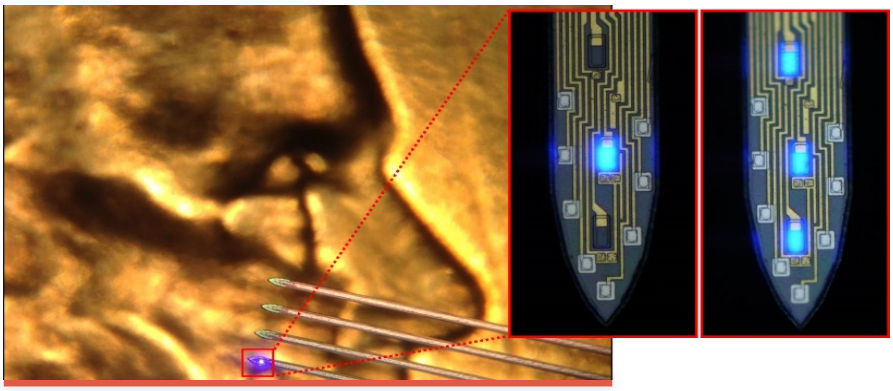

Image: Microphotograph of a tip of a miniSTAR optoelectrode, on which eight recording electrodes, three LEDs, LED interconnects, shielding layer, and recording electrode interconnects are shown. Credit: University of Michigan, Yoon Lab.

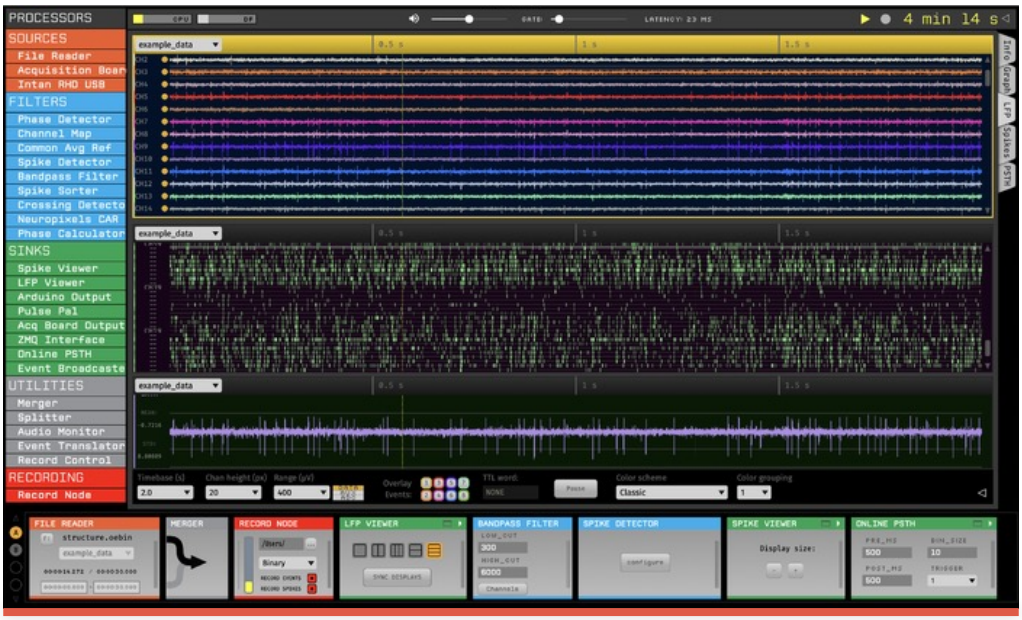

Image: The Open Ephys GUI, modular, open-source software for extracellular electrophysiology. Credit: Open Ephys, 2022.

On the front cover: Top Right Hexagon: An image still from a video of the *C. elegans* brain, including every nerve and muscle fiber, being reconstructed by serial-section electron microscopy. Credit: Daniel Witvliet, University of Toronto and Harvard University, 2020. **Top Central Hexagon:** Four-week-old rat cortical neurons labeled for dendrites (red), axons (green), and nuclei (blue). Credit: Karthik Krishnamurthy, Davide Trotti, Piera Pasinelli, Thomas Jefferson University, 2020. **Bottom Right Hexagon:** A pseudo-colored image of high-resolution gradient-echo MRI scan of a fixed cerebral hemisphere from a person with multiple sclerosis. Credit: Govind Bhagavatheeshwaran, Daniel Reich, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 2016. **Bottom Central Hexagon:** A three-dimensional reconstruction of the entire depth of the front part of the brain (forebrain) of an intact living adult zebrafish imaged with three-photon microscopy (3PEF). The green color indicates very many labeled neurons (small dots), which are expressing a gene for a fluorescent marker of cells. The magenta color shows other features such as the skull and neuronal processes. Credit: NeuroNex: Cornell, 2020.

NeuroNex: Cornell – Dr. Joe Fetcho, Dr. Chris Xu, and Dr. Chunyan Wu

Dr. Joe Fetcho and Dr. Chris Xu of Cornell University study next generation optical microscopy for deeper, wider, and faster brain imaging. Dr. Chunyan Wu, a former graduate student at Cornell, also supported Dr. Fetcho and Dr. Xu in the past. Their goal of rapidly introducing technology advancements to the neuroscience community aligns with that of NeuroNex, a program run by the National Science Foundation (NSF) that has partnered with the BRAIN Initiative to share resources among neuroscience researchers. "Understanding of how the brain works, from the molecular to the systems level, requires interdisciplinary teams of researchers that develop new tools and theoretical frameworks to accelerate discovery and inspire novel concepts and methodologies. The NeuroNex program has helped bring such teams together and support them in their work to advance our knowledge of brain structure and function," said Edda "Floh" Thiels, program director at the NSF. With the help of NeuroNex, the team disseminates optical imaging tools through meetings, campus visits, and commercial vendors.

A few of the tools and technologies that encompass NeuroNex: Cornell include multiphoton microscopy (invented at Cornell), optimum laser exposure, three-photon imaging, and quantitative analysis. The Xu lab recently developed a new technique called 'long wavelength three-photon microscopy' that allows for the deepest non-invasive imaging in animals such as zebrafish and mice. This technique uses special lasers and optical systems to image neurons deep into the brain, making it possible to view the structure of nerve cells and watch individual neurons during behavior.

The Fetcho lab has studied larval and adult zebrafish for over 30 years. With the help of new technologies like long wavelength three-photon microscopy for adult zebrafish and other imaging tools for younger fish, the lab can now follow brain structure and function at all stages of development. This technology helps researchers investigate how developmental disorders of the brain arise and how they differ from other brains. For example, autism can cause genetic differences early in life that influence how the brain functions into adulthood. The research that the Fetcho lab conducts opens the possibility of investigating how drug treatments or genetic manipulation at different stages of life can mitigate brain changes caused by developmental disorders.

As demonstrated by the Fetcho lab's zebrafish research, one of the greatest advantages NeuroNex: Cornell offers is deep and fast imaging. Dr. Fetcho and Dr. Xu compare their deep imaging research to car mechanics — "You cannot look at the hood of a car to figure out how the engine makes it move. You need to know the parts inside and how they connect and interact as well as when they are engaged during the process of making the car move, so you can see how things change when the car stops working correctly."

In the future, the Fetcho lab hopes to image multiple regions of the brain simultaneously to study how different regions interact with each other during behavior. The team is developing new lasers for faster imaging with two- or three-photon microscopy, with new voltage sensors that they hope will give a better view of brain changes associated with brain disorders.

Video: Dr. Joe Fetcho describes how The BRAIN Initiative® has catalyzed collaborations and interactions among scientists and other labs to gain a better understanding of the brain.

> "New tools produced by other labs with support of the BRAIN Initiative are leading to better sensors that allow monitoring of the electrical events themselves in single nerve cells."
>
> *— Dr. Joe Fetcho*

NeuroNex: Bioluminescence Hub – Dr. Christopher Moore

Another resource made possible by NSF's NeuroNex program is the Bioluminescence Hub, run by principal investigator Dr. Christopher Moore and hosted by Brown University, Central Michigan University, and the University of California San Diego. The Bioluminescence Hub investigates new ways to use bioluminescence for optimal brain control and imaging by building and distributing tools and hardware that control cells and track activity. Dr. Moore's goal for the Hub is to support new methods and related technologies across neuroscience and educate high school and undergraduate students with them.

> "Optical synapses targeted to specific circuits impacted by disease could be used as a therapeutic."
>
> — *Dr. Christopher Moore*

The Bioluminescence Hub's suite of tools include molecular constructs, viral vectors, imaging instrumentation, and experimental protocols. These tools allow researchers to conduct imaging with improved signal-to-noise ratio over fluorescence imaging. They have also led to advances like discovering new molecules, amplifying imaging brightness or speed, achieving real-time optical synapse, developing new strategies for conferring calcium sensitivity, and targeting presynaptic luciferases.

One of the tools offered by the Bioluminescence Hub and being studied by Dr. Moore's lab is a technique called bioluminescence-driven optogenetics, or BL-OG. With BL-OG, bioluminescent light drives optogenetic sensors for cell-specific neural circuit stimulation in mice. BL-OG is done using LuMinOpsins (LMOs), molecular constructs that can attach a light producer (luciferase) to photosensory proteins like opsins. BL-OG makes research more flexible and trackable because it allows non-invasive chemogenetic and optogenetic access simultaneously within the same neuron. In 2021, *Cell* detailed some of the protocols and customization strategies the Moore lab uses for BL-OG.

In the future, the Bioluminescence Hub will continue the dissemination of bioluminescence as a neuroscience research tool through workshops, emissaries, symposia, and by providing access to new research developments.

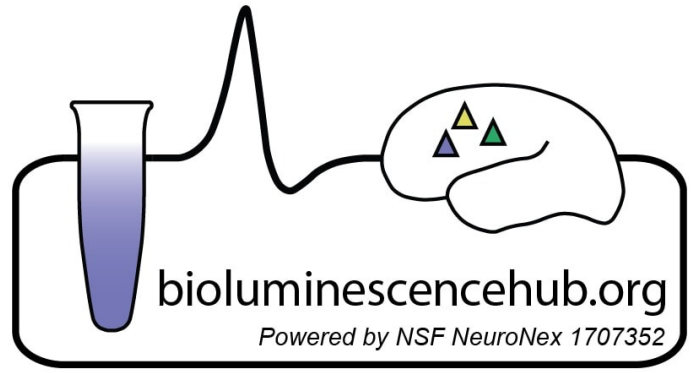

Image: NeuroNex: Bioluminescence Hub logo. Credit: The Bioluminescence Hub, 2022.

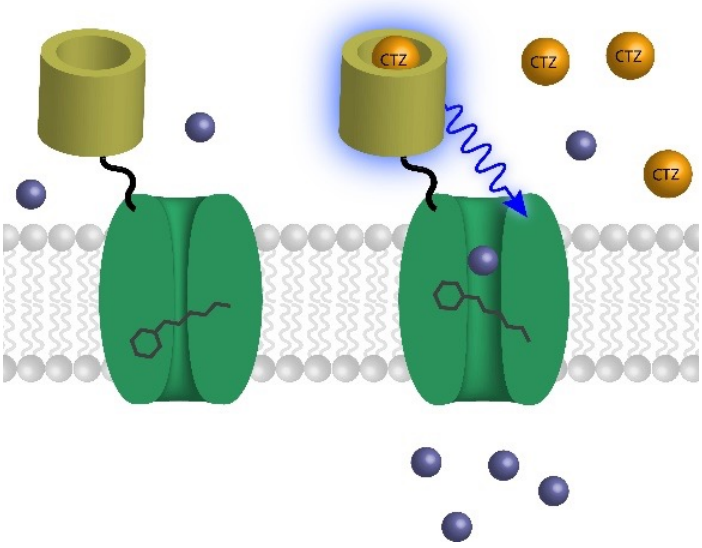

Image: Bioluminescent optogenetics, the use of bioluminescent light to drive optogenetic sensors, can be achieved with molecular constructs called LuMinOpsins (LMOs), where a luciferase (yellow) is tethered to a channel (green) or pump (not shown). When the luciferin (orange) is present, the resulting bioluminescent light is sensed by the opsin (black), opening the channel or activating the pump. Credit: The Bioluminescence Hub, 2022.

Open Ephys Graphical User Interface (GUI) – Dr. Josh Siegle

The Open Ephys GUI is an open-source, plugin-based, cross-platform application that Dr. Josh Siegle of the Allen Institute in Seattle, WA, has worked with for nearly a decade. The software allows neuroscientists to easily acquire and visualize data from thousands of electrodes implanted in the brain. The Open Ephys GUI is funded in part by the National Institutes of Health (NIH) BRAIN Initiative's Dissemination Program.

Dr. Siegle and Dr. Jakob Voigts (now a Group Leader at the Howard Hughes Medical Institute's Janelia Research Campus) co-founded Open Ephys in 2014 with the goal of centralizing electrophysiology development efforts. Open Ephys showcases existing open-source tools that deserve wider recognition, while also building new tools that work within a flexible, modular ecosystem. The development of the Open Ephys Graphical User Interface (GUI) began in Dr. Matt Wilson's lab at the Massachusetts Institute of Technology, as an attempt to replace their decades-old electrophysiology rigs with a modern, open-source alternative. The software was designed in tandem with a mass-producible "acquisition board" that was met with high demand. To ramp up production and distribution efforts, Dr. Siegle partnered with the Open Ephys Production Site in Lisbon, Portugal, to deliver over 800 Open Ephys acquisition boards throughout the field. The acquisition board is the main piece of hardware that is compatible with the Open Ephys GUI, which also works with Neuropixels probes and data acquisition systems from Intan Technologies.

eLife (2014) was the first publication to highlight the Open Ephys GUI's closed-loop feedback capabilities. The authors used the GUI's "Phase Detector" plugin to deliver optogenetic stimulation at precise phases of the hippocampal theta rhythm. Today, hundreds of labs across the world are using the Open Ephys GUI to acquire electrophysiology data from the full range of Open Ephys tools. The GUI's plugin architecture allows users to mix and match data processing modules to build signal chains that are optimized for their specific experiments. "The NIH BRAIN Initiative's U24 Dissemination Program has provided the support to enable these investigators to expand access of Open Ephys software, beyond a single institution, to many among the Neuropixels community. These types of dissemination efforts are a priority goal of the BRAIN Initiative," describes Margaret (Meg) Grabb, program director at the National Institute of Mental Health.

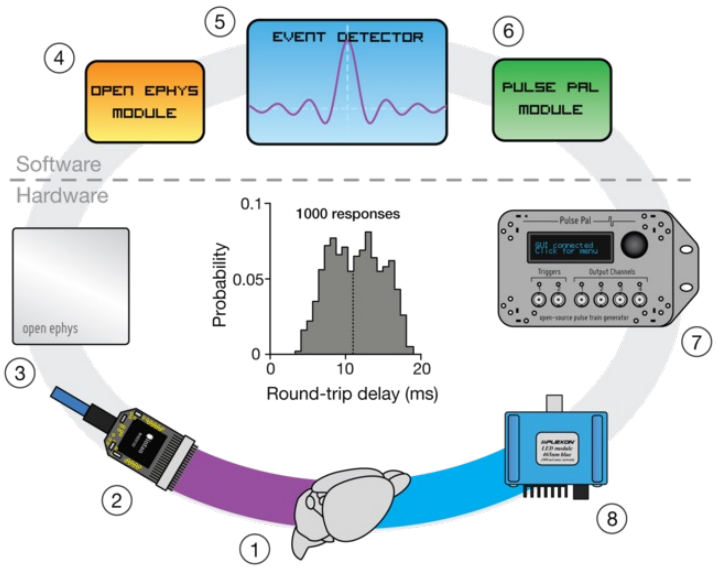

Image: An example of how Open Ephys tools can be used to deliver closed-loop feedback to the brain. The central plot shows a histogram of the total amount of time elapsed between an event occurring and the feedback being delivered. Credit: Open Ephys, 2022.

> "Recently, we created an Open Ephys GUI plugin for Neuropixels probes, which means it can be used by the more than 650 labs that have purchased these probes so far."
>
> — *Dr. Josh Siegle*

If you are interested in learning more about Open Ephys GUI, Dr. Siegle recommends checking out the Open Ephys GUI User Manual. The manual features everything a researcher could need to get started with Open Ephys GUI, including hardware requirements, installation instructions, and documentation about the supported plugins. You can also find out if there are any Open Ephys GUI users in your area by sending a message to info@open-ephys.org.

A full list of publications and preprints based on data collected with the Open Ephys GUI is available at open-ephys.org/publications.

Michigan µLED Probes – Dr. Euisik Yoon and Dr. Roberto Lopez

At the University of Michigan, Dr. Euisik Yoon and his team are developing high-density recording, minimal-stimulation-artifact (miniSTAR) optoelectrodes that integrate microscopic light sources (µLEDs) onto silicon probes. When paired with optogenetics, this tool allows neuroscientists to use light to control the electrical activity of neurons with unprecedented precision in the freely behaving animals.

The Michigan µLED probes are currently being disseminated by the NeuroNex Michigan Hub, Multimodal Integrated Neural Technologies (MINT), to the neuroscience community, to tackle challenging questions that require the precise modulation of specific neuronal cell populations while recording from up to hundreds of neurons at a time.

MiniSTAR optoelectrodes also solve many challenges presented by the optical fibers that neuroscientists relied on until recently, such as damage to the brain area, poor reproducibility, and poor spatial control. MiniSTAR optoelectrodes reduce damage to brain tissue because the microscopic light sources are embedded onto the device itself. "Furthermore, these microscopic light sources are located right next to the recording contacts, allowing the delivery of light exactly where we need it," says Dr. Roberto Lopez, the Program Manager of the MINT. "The µLEDs can produce high illumination levels, matching what can be achieved with optical fibers, from optical tagging and local modulation of neurons to controlling behavior."

The first publication on the Michigan µLED probes appeared in 2015 in *Neuron*, outlining how the researchers created the µLED probes on silicon shanks to control distinct cells in mice, and how the technology would be scalable. Since then, the team at the University of Michigan has used the µLED probes for multiple cell type manipulation, real-time event detection, and closed-loop neural circuit perturbation. The team is also working on refining high density optoelectrodes to scale up to 128 recording sites and 64 µLEDs. This would allow neuroscientists to scale up their research by recording from and controlling larger areas of the brain with the same high temporal resolution and spatial precision.

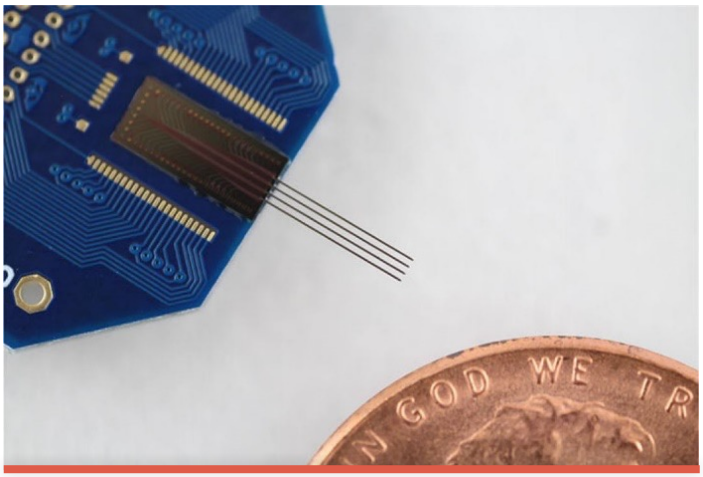

Image: A miniSTAR optoelectrode mounted on a printed circuit board. Credit: Kanghwan et al., 2020, *Nature Communications*.

More details about the lab's recent developments in scaling can be found in *Advanced Science* (2022). For more information about the Michigan µLED probes, contact the University of Michigan's Yoon Lab.

> "Looking a little bit more into the future we are planning on scaling even further the reach of these devices, by adding more versatility in terms of configuration and functionality."
>
> — *Dr. Roberto Lopez*

Excited by the potential of the tools in this issue?! Stay tuned for our next issue and explore more products of BRAIN Initiative discoveries in our Toolmakers' Resources page!