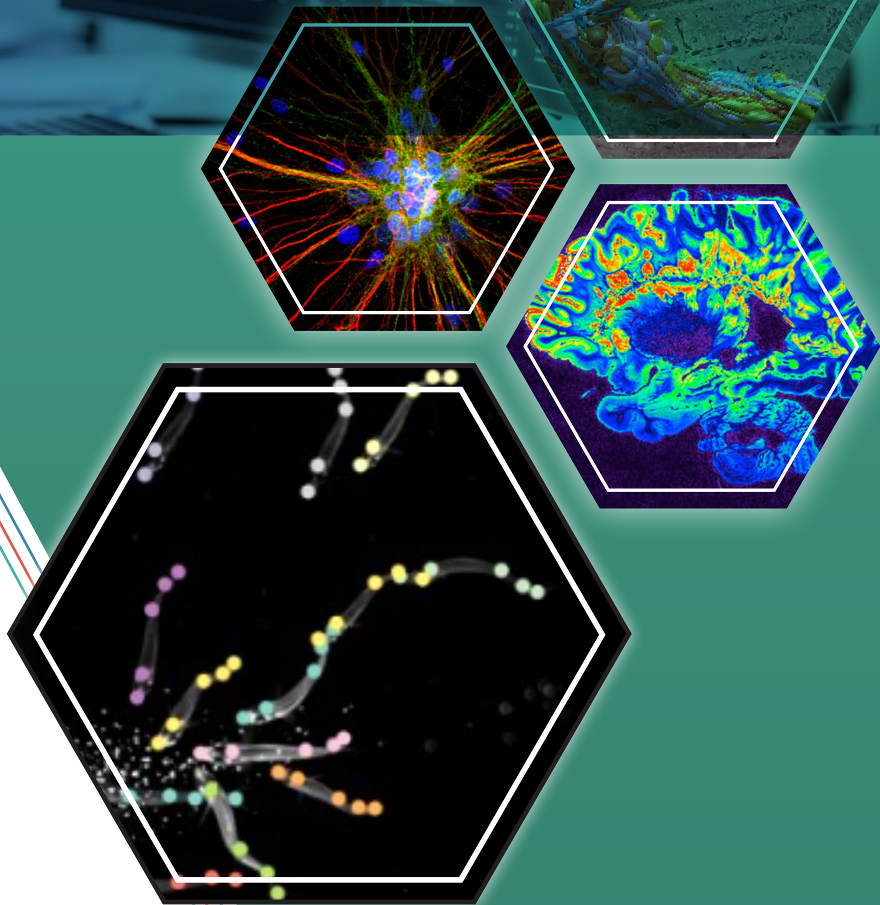
MAY 2023

Toolmakers Newsletter



ISSUE 10

In this issue

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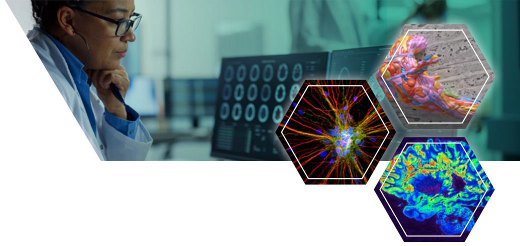


Welcome Back to the Toolmakers Newsletter!

Welcome to the second *Brain Research Through Advancing Innovative Neurotechnologies*® (BRAIN) Initiative Alliance Toolmakers Newsletter of 2023!

In this issue, we are excited to highlight new advancements and capabilities of four tools: 3D Multi-Electrode Arrays by Dr. Samuel Sober; NeuroTools by Dr. Kimberly Ritola; the Brain Image Library by Alexander Ropelewski and Dr. Alan Watson; and DeepLabCut by Dr. Alexander Mathis and Dr. Mackenzie Mathis. Let's learn more details about these projects and some insights from the investigators!

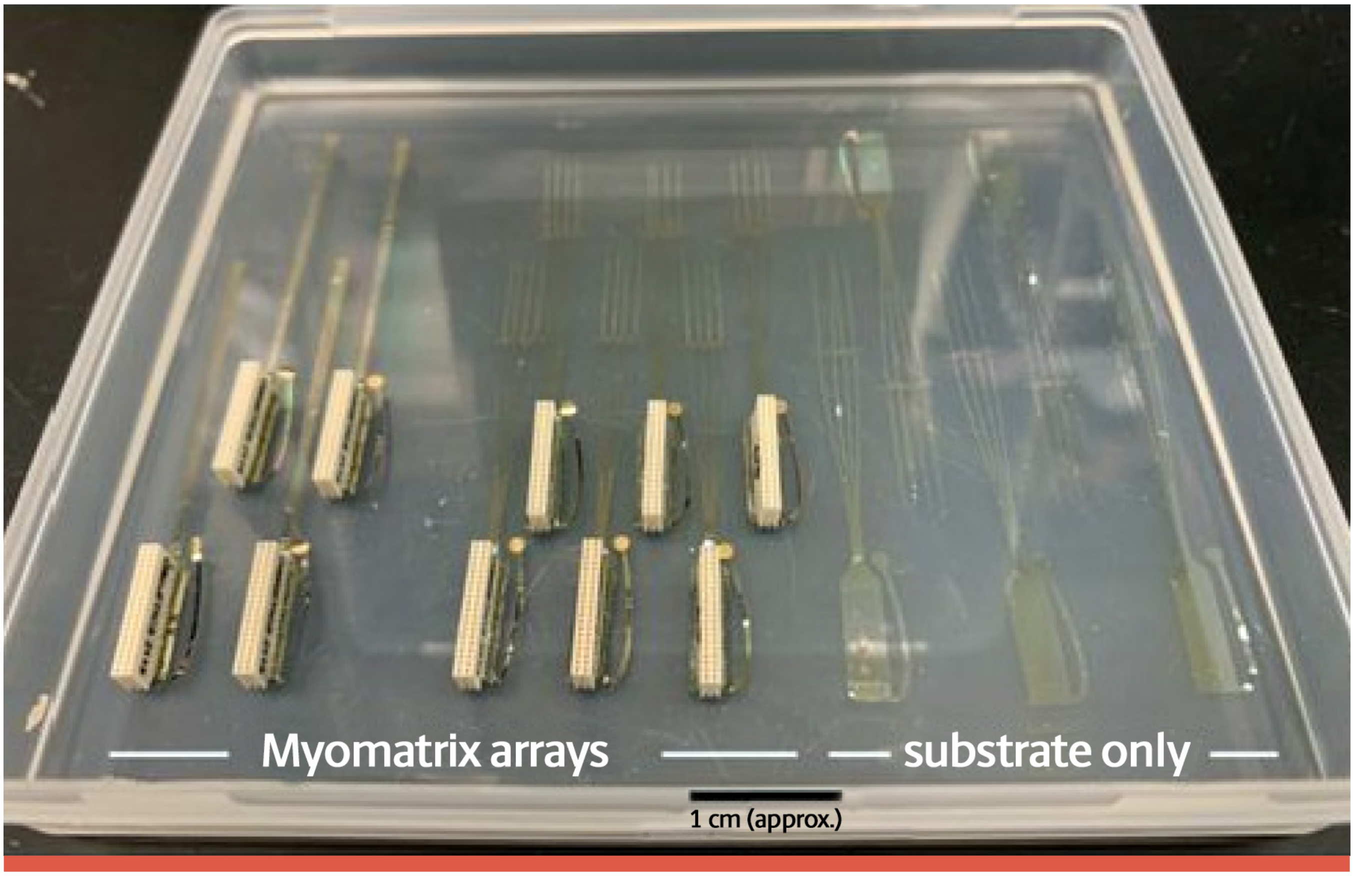


Image: Photo showing two different Myomatrix designs (left) as well as "blank" arrays comprised only of the flexible polyimide substrate for surgical practice and design optimization. Credit: Chung et al., 2023, *bioRxiv*.

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:** Example (cropped) image with (manual) annotations for a dataset containing schooling fish. Credit: Lauer et al., 2022, *Nature Methods*.

3D Multi-Electrode Arrays – Dr. Samuel Sober

At Emory University, [Dr. Samuel Sober and his lab] developed a new technology for examining the signals that the brain uses to control the body. These electrodes ("[myomatrix arrays]"), developed in collaboration with [Dr. Muhannad Bakir's] group at Georgia Tech, record electrical activity in muscles at single-cell resolution, revealing the brain's control of the body in unprecedented detail and skilled movements. Dr. Sober and colleagues have used these electromyography (EMG) electrodes to obtain high-resolution recordings from different muscle groups in songbirds, mice, rats, frogs, and non-human primates.

Dr. Sober's lab studies behaviors such as how songbirds use respiratory muscles to produce vocalizations and how mice use their forelimbs for natural movement. Each recording provides a high-resolution measurement of an individual motor unit, comprised of a single motor neuron and the muscle fibers it activates. Other applications of this technology include recording muscles' bulk activity (rather than single motor units) to examine the total activation of a single muscle. Using that recording data, the lab investigates spiking activity in muscle tissues to uncover the brain signals that control movement.

These multi-electrode arrays are highly stable, dense, and flexible technologies for recording motor outputs. They can isolate the activity of individual motor units during active movements, in different muscle groups, and from a large variety of species. "Our technology can be applied to any muscle group with only minor adjustments to the electrode array design," says Dr. Sober. "We have already used our devices in muscles controlling locomotion, reaching, breathing, facial movements, and other behaviors."

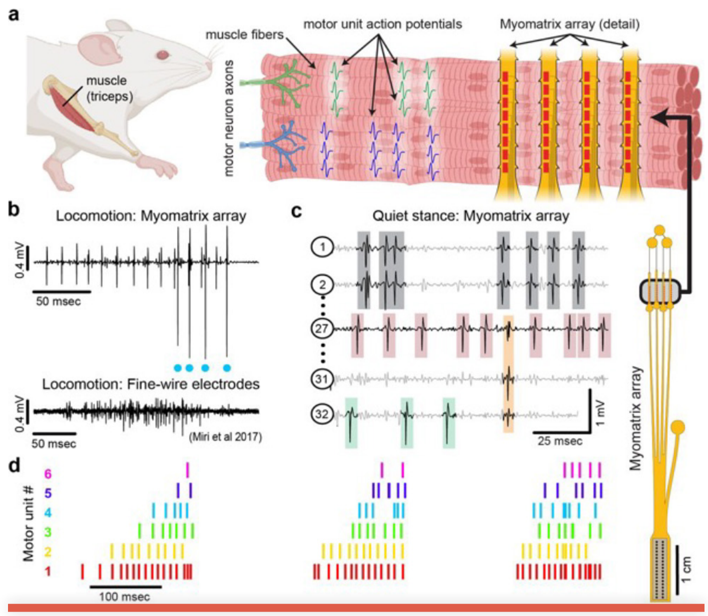
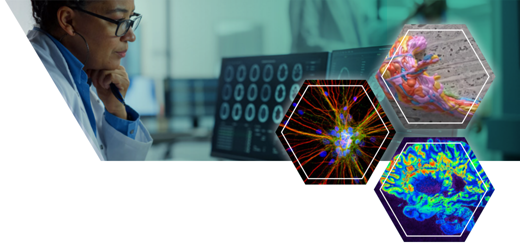


Image: Myomatrix arrays record muscle activity at motor unit resolution. Credit: [Chung et al., 2023, *bioRxiv*].

A recent preprint in *[bioRxiv]* highlighted the Sober lab's myomatrix arrays, which promise maximum stability while recording isolated motor units during active movements and behaviors, whereas other EMG methods can typically only measure isometric actions. These arrays are also small enough for dozens of channels to record from a single muscle, and they provide high-resolution recordings of the nervous system's motor outputs.

The next step for the Sober lab's work includes an upcoming grant that will allow the lab to expand access to their technology to the global neuroscience community. In the future, they want to continue expanding their capabilities for high-resolution recordings in humans.

> "By creating new flexible, microscale electrode devices, we are now able to record single-unit spiking activity in muscle tissue during active movements, which provides us with previously unavailable access to the signals that the brain and spinal cord use to control movement."
>
> *– Dr. Samuel Sober*



NeuroTools – Dr. Kimberly Ritola

The NeuroTools Vector Core is a service led by Dr. Kimberly Ritola and her team at the University of North Carolina (UNC) Neuroscience Center that guides investigators toward the most ideal viral vectors they may need for neuroscience research. The service is still relatively new having begun at UNC less than two years ago, but it is able to help unique experiments work thanks to the high quality of the viral vectors and the resourcefulness of and collaboration by the NeuroTools team.

Image: The NeuroTools logo. Credit: NeuroTools, 2023.

NeuroTools offers a few different types of viral vectors used for neural circuit marking, mapping, and manipulation. Adeno-associated virus (AAV) vectors can be tailored with exact payloads and capsid combinations. NeuroTools specializes in small volumes (100-500 microliters) that specifically cater to the needs of neuroscience experiments. Other vectors also include rabies, lenti-virus, and Herpes simplex virus (HSV) vectors, which allow scientists to choose exact payloads with the exact envelope protein that best suits their needs. Strong collaborations with Dr. Rachael Neve have progressed with the NeuroTools team now leading production on HSV vector development. Dr. Ritola notes that Dr. Neve was "exceedingly generous and supplied us with all the reagents and supplies we would need to carry on her critical work."

Customizability is one of the greatest advantages of NeuroTools, and custom offerings are done affordably with quick production rates. The NeuroTools team is very familiar with the specificities of vector preparation in experiments that involve spinal cord injections, neonates, non-human primates, and crossing the blood-brain barrier. The team also provides full support for ordering and using their viral vectors. They work directly with scientists from consultation discussions to performance troubleshooting. If one of their vectors is not working because of experimental constraints, they help adjust the prep to optimize its features or delivery characteristics. They can also create new payloads or adjust titer or purity requirements as necessary—one of the features that makes the NeuroTools protocols differ from larger vector cores.

NeuroTools prioritizes viral vector efficacy to help ensure study reproducibility and exists as a resource for scientists to learn and experiment with vectors to determine what works best for specific neural regions. The NeuroTools team continuously works to improve their vectors and provide quality assurance to help serve the broad neuroscience community. Investigators interested in using NeuroTools or placing an order should register for a NeuroTools account to gain access to the NeuroTools formal request system.

> "We can adjust our protocol to get sufficient titers for AAV expression vectors up to 6.5 kilobases. This is an important area we continue to investigate how to improve packaging efficiency, vector genome integrity, and most importantly, biologically relevant expression levels."
>
> *— Dr. Kimberly Ritola*

Brain Image Library –Alexander Ropelewski and Dr. Alan Watson

The Brain Image Library (BIL) is a national public neuroscience resource that stores massive datasets so that scientists can upload, visualize, analyze, and share large amounts of fluorescent brain microscopy data. The BIL is a partnership between the Biomedical Applications Group at the Pittsburgh Supercomputing Center and the Center for Biological Imaging at the University of Pittsburgh.

The scope of the BIL's data includes over 7,000 datasets that range from gigabytes to hundreds of terabytes each. Much of this data is whole brain microscopy image datasets, but it also includes partial datasets, targeted experiments, and historical microscopy collections. Additionally, the repository can support unlimited amounts of data in each dataset. The BIL has petabytes of primary imaging data from the highest-resolution microscope types and data from spatial transcriptomics and multi-modal experiments. As of now, it contains more than 133 million public files of data.

A resource like the BIL is essential for the neuroscience community because it offers a central place for scientists to archive and share data in high detail at the cellular and molecular levels. The data collections in the BIL "have significantly greater value than the individual data, as a resource for understanding principles of neural connectivity across brain regions, circuit architectures, and individual variation," says Alexander Ropelewski, a principal investigator at the Pittsburgh Supercomputing Center. "Being long-term stewards of data, we strive to make BIL data FAIR (findable, accessible, interoperable, and reusable), ensuring the data are easy to find and with limited usability barriers to promote reuse of the data." The BIL's datasets meet rigorous metadata standards to help keep them FAIR and meet the standard data sharing policies of neuroscience funding agencies and publishers.

Researchers interested in storing or using data in the BIL should check out the BIL's website to learn more about the Library's ecosystem. The BIL team also offers free trainings and workshops periodically on data submission and exploration. Lastly, the BIL has a helpdesk for any questions or to discuss data needs and scope, including online office hours each month.

> "We expect to grow greater than an order of magnitude over the next few years as greater emphasis is placed on acquiring human and non-human primate data and the BRAIN Initiative Cell Atlas Network (BICAN) data generation efforts begin."
>
> — *Dr. Alan Watson,*
> *Principal Investigator, University of Pittsburgh*

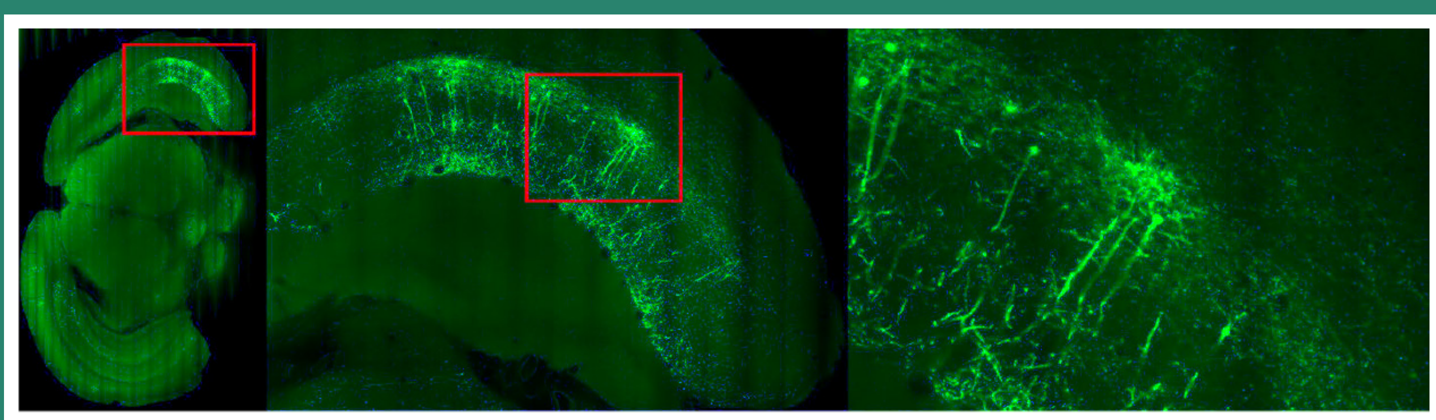
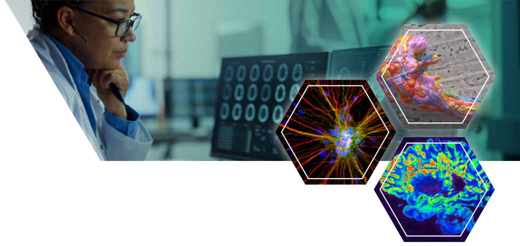


Image: Example fluorescence micro-optical sectioning tomography (fMOST) dataset archived at the Brain Image Library displaying viral tracer injection. Data were derived from Dr. Hongkui Zeng's group and the Allen Institute for Brain Science. Credit: Brain Image Library, 2023.



DeepLabCut – Dr. Alexander Mathis and Dr. Mackenzie Mathis

DeepLabCut is a deep-learning tool that provides markerless motion capture to help understand the science behind behavior in animals like flies, fish, mice, rats, horses, cheetahs, and primates, including humans.

The software was first published in *Nature Neuroscience* in 2018, where it gained attention for its unique ability to automate movement tracking. Not only does it make behavioral monitoring easier, but it can reliably do it across species that move in different ways. It can conduct movement analyses, study social behaviors, and track facial keypoints. Since then, DeepLabCut has become the go-to method for animal tracking and quantifying behavior at hundreds of labs.

> "My favorite aspect of DeepLabCut is how it enables researchers without coding experience to tackle biological questions that they are interested in."
>
> *— Dr. Alexander Mathis*

DeepLabCut, led by Dr. Alexander Mathis and Dr. Mackenzie Mathis, recently turned five years old, and over the years has had a team of collaborators from the University of Tübingen, Harvard University, the Massachusetts Institute of Technology (MIT), and École Polytechnique Fédérale de Lausanne (EPFL). Currently, the team at EPFL develops the code for DeepLabCut, and many other scientists globally help contribute to and actively develop and maintain it through GitHub and ImageForum SC.

One of the newer developments for DeepLabCut involves improving pose tracking methods in crowded environments, such as when multiple animals are moving and interacting closely together. Dr. Catherine Dulac, Dr. Venkatesh N. Murthy, Dr. George Lauder, and Dr. Guoping Feng contributed data to help benchmark the development of this multi-animal version of DeepLabCut, which was published in *Nature Methods* last year. "Furthermore, we are integrating the capabilities with other packages such as CEBRA for jointly analyzing behavior and neural data," says Dr. Alexander Mathis, Assistant Professor at EPFL.

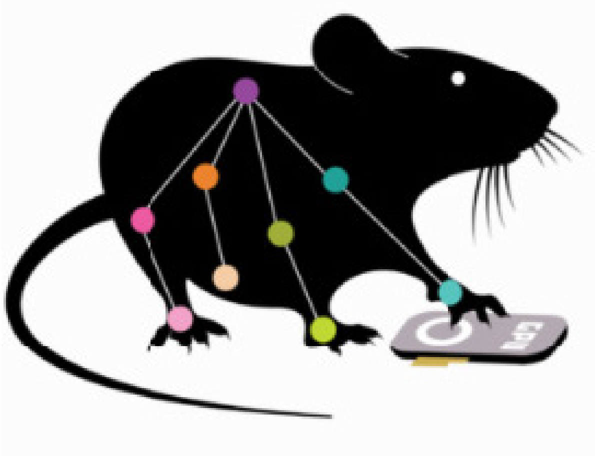


Image: DeepLabCut logo. Credit: Dr. Mackenzie Mathis, 2023.

With nearly 500,000 downloads, DeepLabCut team is always looking to improve their technology and expand the software's use within the scientific community. In the future, the team would like to grow their user base and expand the application domains.

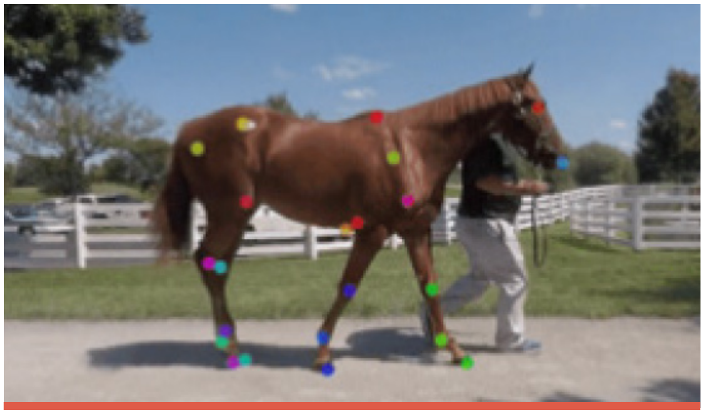


Image: DeepLabCut helps track various body parts for animal behavioral analysis, as demonstrated here with a horse walking. Credit: Mathis Group, 2023.

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!