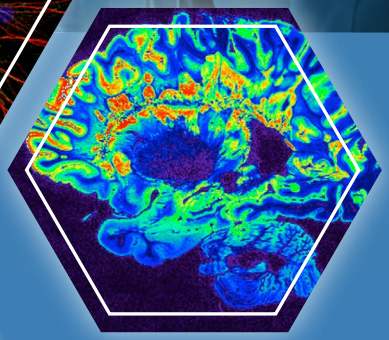
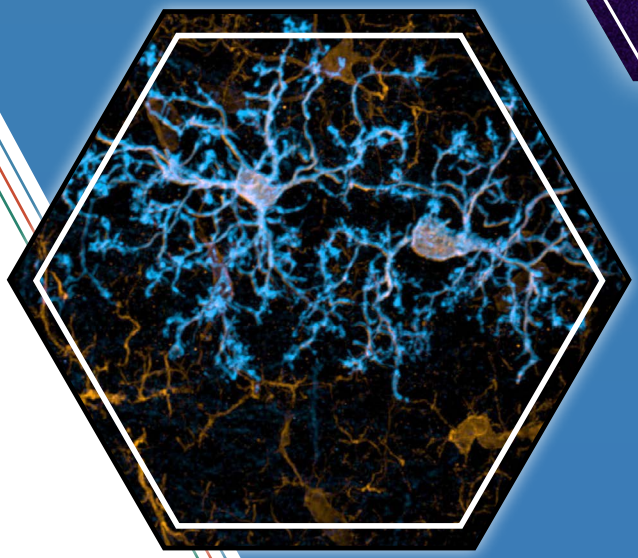
FEBRUARY 2023

Toolmakers Newsletter





ISSUE 09

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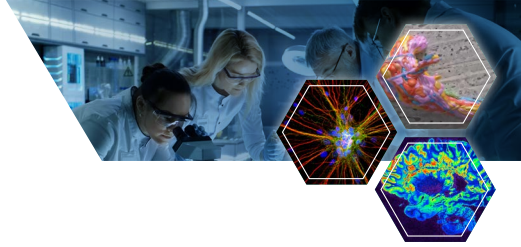


Welcome Back to the Toolmakers Newsletter!

Welcome to the first *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: the Two-photon Spectrometer by Dr. Mikhail Drobizhev; Soft Multifunctional Neural Probes by Dr. Siyuan Rao; Distributed Archives for Neurophysiology Data Integration (DANDI) by Dr. Satrajit Ghosh; and MOnonucleotide Repeat Frameshift (MORF) Genetic Sparse Cell Labeling by Dr. X. William Yang. Let's learn more details about these projects and some insights from the investigators!

Image: A hydrogel microelectrode. Credit: Rao Lab.

On the front cover: **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:** Brainwide Genetic Sparse Cell Labeling to Illuminate the Morphology of Neurons and Glia with Cre-dependent MORF Mice. Credit: Veldman et al., 2019, *Neuron*.

Two-photon Spectrometer – Dr. Mikhail Drobizhev

The two-photon spectrometer is a resource by Dr. Mikhail Drobizhev and his team at Montana State University that guides two-photon laser microscopy (TPLM)—an imaging technique that uses near-infrared femtosecond lasers for deeper, wider, and faster brain imaging. The resource helps researchers quantify two-photon excitation of fluorescent proteins to learn what proteins are the brightest and to determine what are the best excitation laser wavelengths.

To use TPLM to its full potential, researchers must know the two-photon absorption spectra of fluorescent probes (to select the best laser wavelength), their cross sections (to understand the structure—property relations in a set of homologous proteins), and their two-photon brightness (to help determine which proteins are brightest upon two-photon excitation). The two-photon spectrometer can quickly characterize these parameters, allowing comparison of different fluorescent probes' photostability so that researchers can choose the brightest and most stable probes possible. This knowledge will make it possible to deeply image neurons non-invasively with high spatial resolution.

> "Our main goal is to help protein engineers and synthetic chemists to create or find the best fluorescent probes and optimize laser parameters for using them in multiphoton microscopy."
>
> *– Dr. Mikhail Drobizhev*

One of the greatest advantages of the two-photon spectrometer is its characterization abilities—two-photon characterization helps compare probes to maximize excitation efficiency. "Using two-photon brighter probes with optimum excitation wavelengths helps to reduce laser power, and consequently remove detrimental thermal heating of neurons," says Dr. Drobizhev, "This, in its turn, allows longer imaging time for obtaining more information on dynamic processes in neurons."

Careful characterization also enables a multicolor approach when imaging processes located deep inside of tissues. Different fluorophores with varying wavelengths can be excited with a single laser wavelength by using genetically encoded probes and sensors.

Dr. Drobizhev's lab aims to provide researchers with information about the multiphoton properties of new probes and sensors and guide them through how to genetically modify them to create new variants with stronger two-photon absorption or larger fluorescence response to Ca^{2+} or other messengers of neuron activity. This guidance is based on the structure-property relationships that were established by studying the role of protein electrostatics in photophysical properties of fluorescent proteins.

This resource helps researchers find the optimal laser excitation wavelengths for probes, optimize two-photon brightness and photostability, and improve the dynamic range of genetically encoded sensors. So far, 17 different labs in 6 countries are using the two-photon spectrometer. The scope of the probes incudes traditional genetically encoded fluorescent proteins with the GFP scaffold, new near-infrared fluorescent derivatives of phytochromes, and chemically encoded proteins based on HaloTag technology.

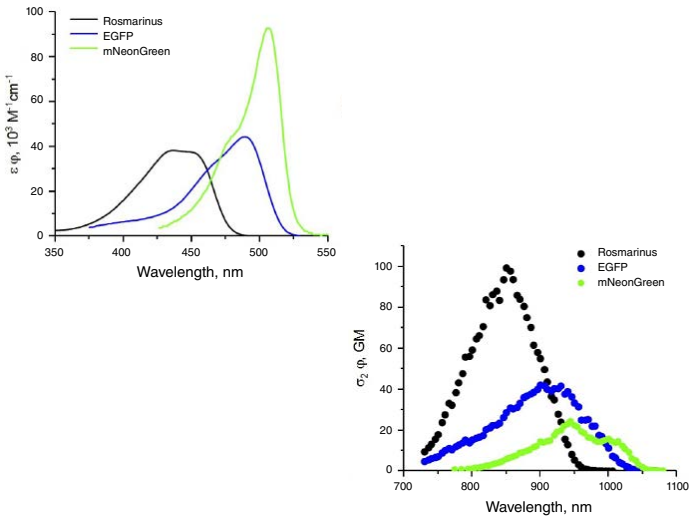
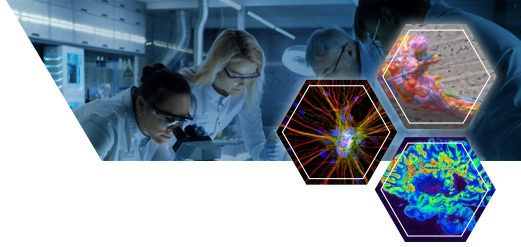


Image: The two-photon absorption brightness cannot be predicted from the one-photon counterpart. The left panel shows the one-photon excitation spectra of three selected fluorescent proteins, and the right panel – their corresponding two-photon spectra. As one can see, mNeonGreen (green curve) is exceptionally bright in the 1P regime (left) but fairly dim in 2P (right), as compared to enhanced green fluorescent protein (EGFP) (blue curve) and the GFP homologue Rosmarinus (black curve). Credit: R.S. Molina et al., 2017, *J. Phys. Chem*.



Soft Multifunctional Neural Probes – Dr. Siyuan Rao

At the University of Massachusetts Amherst, Dr. Siyuan Rao and her lab have been developing soft multifunctional neural probes for over two years. The probes are part of a toolset used to study neural activity using external devices for things like optogenetics and electrophysiological recording in conjunction with behavioral testing.

The soft multifunctional neural probes are unique because they are made of crosslinked hydrogels that are biocompatible and viscoelastic—they are soft, highly transparent, elastic, and compatible with the optical properties of mice and rats. Because they are so adaptable, they are used as optical probes to study visual behavior with minimal tissue damage.

> "The strong support from NIH, BRAIN Initiative, and other agencies are encouraging my young research lab and myself to continue our endeavor to develop next-generation effective neuroengineering tools for broad communities."
>
> *– Dr. Siyuan Rao*

Dr. Rao's lab is using the neural probes in optogenetics research to study bi-directional optical interrogation through multiple modalities. "Our current generation of neural probes can record optical and electrical signals from the optical core and microelectrodes within the same device independently," said Dr. Rao. Microelectrode integration is a big advantage of this technology—the team invented hydrogel microelectrodes that are very thin, soft, and stretchable so that they can be placed outside of the optical core without affecting any light transmission or the core's structure. The optical core is made from high-refractive index hydrogels to transmit light efficiently and monitors fluorescence intensity changes in ion indicators expressed in neurons, while the microelectrodes record electrophysiological signals independently.

Some of the greatest benefits of the soft multifunctional neural probes are their functionality. They are adaptable and their mechanical properties can be adjusted in diameter for maximum precision. To create the probes, the lab doesn't require any advanced facilities—they are accessible and can be custom molded or extruded. The probes only take seconds to prepare meters of hydrogel fibers that are long-lasting. When combined with other functional components or interfaces, the probes can help researchers achieve localized drug and virus delivery in hopes of investigating therapeutic advances for neurological or psychiatric disorders.

Image: Soft multifunctional neuro probes wrapped around a finger. Credit: Rao Lab.

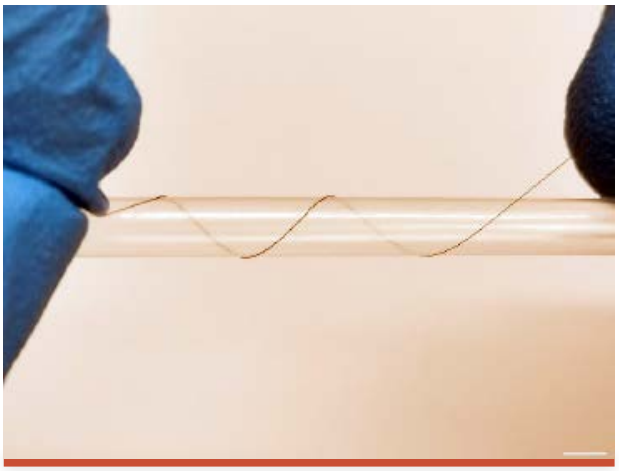


Image: A hydrogel microelectrode. Credit: Rao Lab.

Distributed Archives for Neurophysiology Data Integration (DANDI) –Dr. Satrajit Ghosh

DANDI is a data archive that is home to 233 neurophysiology datasets and 440 terabytes of data. The platform supports data storage, searching, viewing, analysis, and publishing through "Dandisets," the collection of files gathered from a project.

> "The public data in DANDI is openly accessible to anyone, while embargoed data are accessible to any collaborators (and will become public when brought out of embargo). We facilitate storing, disseminating, and computing on large, terabyte sized Dandisets."
>
> *– Dr. Satrajit Ghosh*

DANDI is most commonly used to support existing publications, but as the archive grows, the DANDI team is seeing additional uses. "For example," says DANDI team member Dr. Satrajit Ghosh, "a recent project by Sabera Talukder in the Yue Lab at CalTech used the AJILE12 Dandiset, containing human longitudinal electrocorticography (ECoG) recordings, to build an inference algorithm that can impute missing or noisy ECoG data." This project was recently preprinted in *arXiv* and presented at the Conference on Neural Information Processing Systems and "is a strong example of neurophysiology on DANDI being used to fuel scientific and engineering discoveries beyond the scope of the original project."

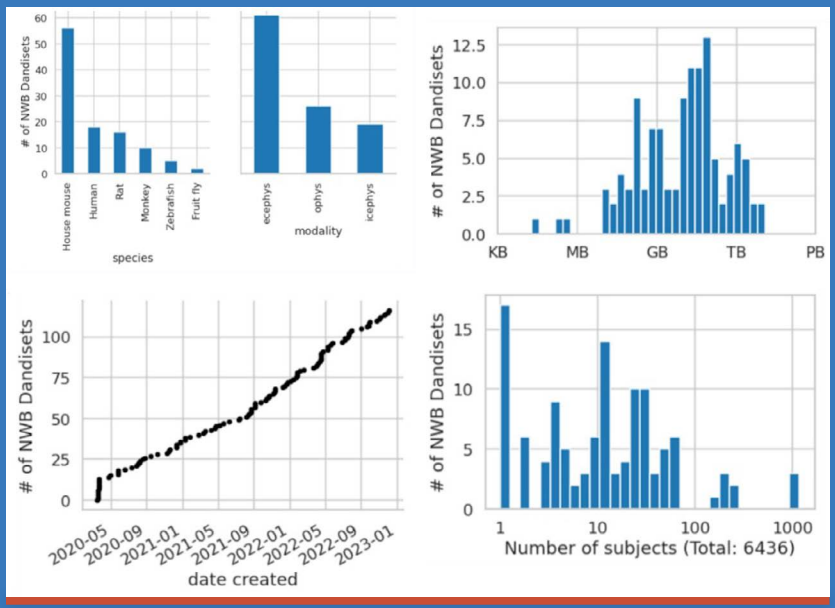


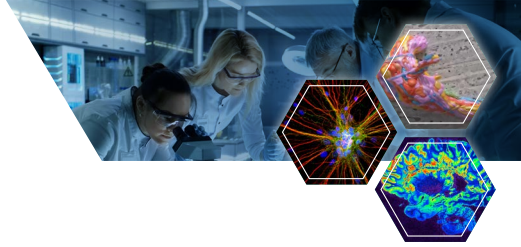
Image: Overall status of the DANDI archive (January 3, 2023). The totals include published, in progress, embargoed data, and the microscopy datasets. Credit: DANDI, 2023.

Image DANDI logo. Credit: DANDI, 2023.

If you checked out our May 2022 newsletter, you may have noticed DANDI referenced in the Neurodata Wtihout Borders (NWB) feature. DANDI requires its neurophysiology data to be in the NWB file format, and the DANDI and NWB teams work together to give users access to large, high-quality datasets. DANDI also supports the Brain Imaging Data Structure (BIDS) and BIDS-like file layouts to help ensure all datasets are compliant with organization and storage standards.

In the future, the DANDI team would like to see the datasets hosted by DANDI being resused to create new, improved tools and technologies. The team hopes that scientists will use DANDI to conduct scientific research by performing data analysis to expedite the research process while complying with FAIR (findable, accessible, interoperable, and resusable) principles. To continue doing this, the team plans to create more advanced applications to help scientists find information faster and increase DANDI's cloud computing infrastructure to automate processes and increase quality control.

Scientists interested in using DANDI for searching data should check out the DANDI Archive's search function, which will match words included in any Dandiset's metadata. Additionally, scientists who want to publish their data on DANDI must first ensure their data is the appropriate form for DANDI. The DANDI Handbook is the best resource for prospective users who wish to harmonize and publish their data.



MOnonucleotide Repeat Frameshift (MORF) Genetic Sparse Cell Labeling – Dr. X. William Yang

MORF is a strategy used for sparse cell labeling of genetically defined populations. At the University of California, Los Angeles (UCLA), Dr. X. William Yang and his lab used MORF to create a scalable method called MORF Genetic Sparse Cell Labeling that develops reporter mouse lines to study neuronal morphology in the mouse brain.

The first publication on this strategy appeared in *Neuron* in 2020, outlining the lab's imaging and analysis efforts to label and illuminate genetically defined neurons. Since then, the team has created a pipeline to help image all MORF-labeled neurons in the brain with automated tissue clearing and immunostaining. This new imaging capability helps analyze single neuron communication and projections from one brain area to another. Over the last few years, Dr. Yang and his team have also improved their bioinformatics capabilities by collaborating with other labs at UCLA to build greatly accelerated, semi-automated tools to help turn brain images into digital, scalable, and shareable neuronal image datasets.

> "We hope that this method will grant neuroscientists the ability to label, image, and quantitatively analyze the morphology and connectomics of genetically defined single neurons and glial cells in intact mouse brains."
>
> *– Dr. X. William Yang*

The team is currently working on evolving the MORF Genetic Sparse Cell Labeling strategy to both label genetically-defined single mouse neurons and investigate their biology more precisely. In what they call "synapse MORF" mice, they selectively label synapses of single neurons, and with "intersectional MORF" mice, the team can study the precise molecular types of single neuron morphology using two genetic drivers. "Together, these new tools will greatly expand the biological questions one can answer at single neuron resolution *in vivo* from synaptic imaging and intersectional labeling to causal genetic perturbations," says Dr. Yang. The new approaches demonstrate how the MORF Genetic Sparse Cell Labeling approach is scalable and can help study *in vivo* neuronal morphological changes to investigate brain diseases and how the brain develops.

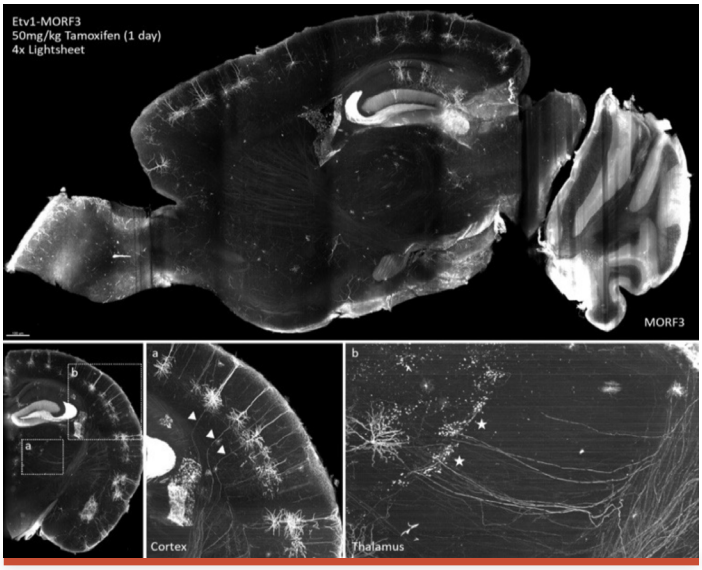


Image: MORF mouse lines confer genetically-directed labeling of brain cell morphology. Credit: X. William Yang Lab, 2023.

The BRAIN Initiative Cell Census Network (BICCN)'s TIGRE-MORF and MORF3 studies are using this cell labeling methodology to profile neuron morphology in thousands of mice neurons in the primary motor cortex. The Yang lab also has ongoing studies that utilize MORF mice to study neurodegenerative disorders like Huntington's disease, Alzheimer's disease, and aging. To learn more about the strategy and related research, visit the MORF website.

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!