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Toolmakers Newsletter

ISSUE 02

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BRAIN

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Welcome Back to the Toolmakers Newsletter!

Welcome back the Brain Research Through to Advancina Innovative *Neurotechnologies*® (BRAIN) Initiative Alliance Toolmakers Newsletter! We are pleased to announce that, thanks to readers like you, our first issue was a resounding success!

In our second issue, we are delighted to report on four more exciting tool advancements: novel applications of miniature micro-endoscopes to record neural activity from multiple brain regions simultaneously by Dr. Walter Gonzalez; new genetically encoded voltage indicators (GEVIs) to monitor the electrical dynamics of individual neurons created by Dr. François St-Pierre; the validation of renewable affinity antibodies and reagents for neuroscience research by Dr. James Trimmer; and cytoNet, a mathematical tool for rapidly characterizing environmental effects on network topology, developed by Dr. Amina Ann Qutub.

Come with us as we explore the latest breakthroughs from these BRAIN investigators.



120 µm

Left Image: Microendoscopic output representing neurons in the hippocampus from 25 sessions analyzed simultaneously in one mouse. Credit: Gonzalez et al., 2019.

Below Image: Time-lapse imaging experiment of HeLa cells expressing a fusion of keratin with mGold (top) or mVenus (bottom). These data show that the mGold YFP has up to fivefold improvement in photostability. Credit: Lee et al., 2020.



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 neural network representation obtained using the cytoNet program. Credit: cytoNet.

Miniature Micro-Endoscope: Recording Brain Activity in Multiple Areas Simultaneously in Mice — Dr. Walter Gonzalez

Dr. Walter Gonzalez, a postdoctoral fellow in the lab of <u>Dr. Carlos Lois</u> at Caltech, leads <u>a team</u> that aims to understand how the activity of individual neurons that comprise neural circuits gives rise to brain functions through the use of custom-built, high-sensitivity <u>miniature micro-endoscopes</u>. These micro-endoscopes allowed Dr. Gonzalez and his team to perform long-term imaging (up to 20 days) of neuronal activity in freely behaving mice to understand how memories form and how they fade. In their <u>seminal paper published in</u> <u>Science in 2019</u>, Dr. Gonzalez and his team demonstrated that these miniature micro-endoscopes can be used to gather meaningful data about important research questions about the longevity of memory.

Right Image: <u>Watch this video</u> of hippocampal CA1 neural activity in one mouse recorded for 8 months (76 sessions). Credit: <u>Gonzalez et al., 2019</u>.



Imagine you have a long and complicated story to tell. In order to preserve the story, you could tell it to five of your friends and then occasionally get together with all of them to re-tell the story and help each other fill in any gaps that an individual had forgotten. Additionally, each time you re-tell the story, you could bring new friends to learn and therefore help preserve it and strengthen the memory. In an analogous way, your own neurons help each other out to encode memories that will persist over time."

— <u>Dr. Walter Gonzalez</u>

Dr. Gonzalez's metaphor paints a picture of how individual neurons come together to form complex networks that have emergent properties such as higher order cognitive processing and memory. Unraveling how memories last and how they fade at the level of individual neurons, as well as in the context of their larger neural circuits, could have incredible consequences for conditions such as Alzheimer's disease. Importantly, the use of these miniature micro-endoscopes may pave the way for a better understanding of memoryrelated diseases thanks to up-and-coming researchers like Dr. Gonzalez.

Molecular Technologies: Genetically Encoded Voltage Indicators (GEVIs) for Monitoring Voltage *In Vivo* — Dr. François St-Pierre

12.85 hrs

At the Baylor College of Medicine, Dr. François St-Pierre is working to create new molecular tools in neuroscience. Specifically, one of Dr. St-Pierre's BRAIN-funded projects is focused on the development and dissemination of new genetically encoded voltage indicators (GEVIs). GEVIs are light-emitting protein indicators whose brightness directly reports voltage and are proving to be promising tools for monitoring voltage dynamics at high spatial and temporal resolution in vivo. His new tool can even use the same wavelengths and equipment as those used in calcium imaging. Dr. St-Pierre made a splash in Science Advances just last year, when he and his team screened approximately 3 million cells expressing mutagenesis libraries. In doing so, they identified a bright new yellow fluorescent protein (YFP) variant. The YFP variant, called mGold, turned out to be the most photostable YFP found to date. Addgene even recently featured mGold in their "Hot Plasmids and Viral Preps" article series. Dissemination of new genetic tools like GEVIs is an integral part of Dr. St-Pierre's research plan. Resources that facilitate collaboration and dissemination, such as those offered by viral repositories, are key to open data and other open science efforts. All in all, Dr. St-Pierre is working to make neuroscience more accessible one plasmid at a time.

Above Image: Time-lapse imaging experiments show the visualization of cell division using mGold. <u>Click here to watch</u>! Credit: <u>Lee et al.</u>, 2020.

20 um





Renewable Recombinant Antibodies and Affinity Reagents for Neuroscience Research — Dr. James Trimmer

At the University of California, Davis, <u>Dr. James Trimmer</u> is working hard to further the mission of neuroscience research by developing, validating, and increasing the neuroscience community's access to a wide range of <u>renewable recombinant antibodies and affinity reagents</u>. As Dr. Trimmer points out on his laboratory's website, while the Human Genome Project has unveiled a wealth of genetic data, ultimately, we have to understand the proteins that those genes code for. Thus, neuroscience researchers need the correct tools that have been optimized for each protein of interest. Specialized tools are increasingly necessary as we strive to understand the exact function of specific cell types, each of which tends to have drastically different structural and molecular complexities. To achieve their tool development goals, Dr. Trimmer and his team created the <u>UC Davis/NIH NeuroMab Facility</u> to develop and facilitate the distribution of thousands of validated antibodies across the neuroscience community. The first set of plasmids encoding recombinant nanobodies are also now available through <u>Addgene</u>. Furthermore, most NeuroMab sequences are also available through the <u>NeuroMabSeq website</u>. This is open science at its finest. The availability of these sequences will allow researchers to generate their own plasmids to create molecularly defined monoclonal antibodies. Check out Dr. Trimmer and his team's latest paper on the development of these tools in *Current Protocols in Neuroscience here!*





Image: Immunofluorescence staining of rat hippocampal neurons with K28/43 (green, PSD-95) and K57/1 (red, Kv4.2). Credit: UC <u>David-NeuroMab</u>.



cytoNet: A Mathematical, Web-Based Tool to Rapidly Characterize Multiscale Networks from Images — Dr. Amina Ann Qutub

Dr. Amina Ann Qutub from the University of Texas at San Antonio recently helped launch a new tool known as cytoNet. The cytoNet software provides a mathematical, web-based approach for rapid spatial characterization of multiscale cellular networks from fluorescence microscope images. More specifically, cytoNet uses graph theory and other mathematical approaches for their cloud-based image analysis software program. In another glowing example of open science, anyone can access this cloud-based program and use it to analyze their microscopy images. Researchers can visit the cytoNet webpage and upload their images. Dr. Qutub's team even provides a <u>user guide</u> and <u>video tutorial</u> to help get researchers started. They also have a <u>recent preprint on bioRxiv</u> that introduces this software to the research community, provides examples of its potential use, and emphasizes that this image analysis framework is accessible to researchers across many domains within the sciences. Keep up the great work, Dr. Amina Ann Qutub!





Click to watch video: Dr. Qutub explains the role of the BRAIN Initiative in the cytoNet project.

Below Image: Spatial neural progenitor cell networks at day 1, 3, and 5 of differentiation, overlaid on immunofluorescence images of nuclei stained with Hoechst dye; segmented cells are outlined in red, and spatial proximity edges are shown as yellow lines. Credit: <u>Mahadevan et al., 2021</u>.



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' Resourcespage!

