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5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS

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7. PERFORMING ORGANIZATION NAMES AND ADDRESSES

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8. PERFORMING ORGANIZATION REPORT NUMBER

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The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Rafael Yuste
UU	UU	UU	UU		19b. TELEPHONE NUMBER 212-854-2354

RPPR Final Report
as of 09-Feb-2022

Agency Code: 21XD

Proposal Number: 61809LSMUR **Agreement Number: W911NF-12-1-0594**
INVESTIGATOR(S):

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DUNS Number: 049179401 EIN: 135598093

Report Date: 30-Jun-2020 Date Received: 28-Jun-2021
Final Report for Period Beginning 01-Oct-2012 and Ending 31-Mar-2020
Title: Imaging How a Neuron Computes
Begin Performance Period: 01-Oct-2012 **End Performance Period:** 31-Mar-2020
Report Term: 0-Other

Submitted By: Rafael Yuste Email: rmy5@columbia.edu
Phone: (212) 854-2354

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STEM Degrees: **STEM Participants:**

Major Goals: During this MURI, the eleven laboratories have developed new imaging methodologies aimed at the scientific goal of understanding how neurons compute with great success. In particular, we have had major publications in the stimulated Raman scattering (Wei and Yuste), methods for methods for demixing spatiotemporal calcium imaging data (Paninski) and their application to multiplane imaging in vivo (Yuste) and sesrial EM studies (Lichtman). We also have continued to develop nanodiamond voltage sensing, atomic force microscopy and SPAD. It is fair to say that this MURI has pioneered, more than any other group, the development of Neurotechnology in the US.

The specifics goals were:

Xiaowei Zhang:
We propose to use STochastic Optical Reconstruction Microscopy (STORM), a super-resolution fluorescence imaging method to determine the synaptic inputs on individual neurons and the connectivity between neurons.

Ken Shepard:
We focus on three ongoing projects in this area:
65,536-channel multielectrode array
Flexible CMOS wireless probes
10,240-channel shank-based electrophysiology recording and stimulating system

Dirk Englund
In this program, we are studying the dependence of the NV-NDs' charge state and fluorescence properties to external voltages. The overarching goal is to use NV-NDs as fluorescent voltage indicators for sensing electrical activity in neurons.

Elizabeth Hillman:
We have focused on two areas:
1)Continued development of SCAPE microscopy for high-speed 3D microscopy in behaving organisms. 2) Development and use of large-scale imaging of neural activity across the awake mouse brain using wide- field optical mapping (WFOM) of GCaMP.

Jeff Lichtman:
The Lichtman Group's goals are:

1.) create the largest volume of brain studied in a connectomic way.
2.) include not only connectivity, but also the ultrastructure of the synaptic connections and the geometry of the neurons.

Rafael Yuste:
Major goals for the Yuste Lab are:
1.) Imaging voltage with genetic, organic and inorganic indicators 2.) Two-photon mapping of synaptic connections

Wei Min:
Develop imaging techniques for small metabolites, including imaging of glucose uptake.

Jonathan Owen:
We are developing voltage sensitive quantum dot (QD) fluorophores for high spatial (1 ?m2) and temporal resolution (100 ?sec) imaging of membrane potential. If successful these would offer the highest performance probe for imaging action potentials in real time and in real space. Probes with these characteristics would allow the subthreshold events at dendritic spines to be mapped and to be analyzed for the first time. We are pursuing this goal in three thrusts: 1) synthesis of voltage sensitive fluorescent QDs, 2) optimizing their structure to increase their sensitivity, and 3) embedding the QDs within membranes using phospholipid surfactant ligands.

Sebastian Seung:
Reconstruction of neural circuits from 3D electron microscopic brain images, using a convolutional network with a mixture of 2D and 3D filters to significantly improve accuracy.

Liam Paninski:
1.) Develop semi-supervised spectral clustering algorithms for segmenting Brainbow images.
2.) Develop new methods to improve the spatial resolution of STORM/PALM superresolution imaging.
3.) Collaboration with Shepard group to develop high-throughput spike sorting and demixing algorithms for large-scale multi-electrode arrays.

Ozgur Sahin:
Dendritic spines are small structures found covering the dendrites of neurons, forming excitatory synapses throughout the brain. Due to their sub-micron size it is very difficult to study the individual synaptic properties of spines using conventional methods such as electrophysiology and optical measurements. We have developed novel methods using Atomic Force Microscopy (AFM) to monitor the electromechanical coupling that occurs in spines during depolarization. AFM experiments on dendritic spines of cultured hippocampal mouse neurons have revealed spontaneous, rapid, mechanical events, which we call spine kicking and seem to result from electromechanical coupling between the cell membrane and AFM probe.

Accomplishments: Elizabeth Hillman:
1) We developed new SCAPE systems with optimized designs. We have developed and optimized the technique with collaborators in a wide range of neural systems. Several publications are in preparation based on this work.
2) We have developed WFOM methods for reliable, longitudinal imaging of awake mouse brain. We demonstrated the use of this technique to understand postnatal development of neural circuits in mouse brain (1). We have utilized the technique to explore the underpinnings of resting state functional connectivity mapping in the mouse brain (in 2nd review (2)). We wrote a detailed tutorial on this WFOM method to enable wider distribution and support of this technique (3). Our low-cost WFOM system design is available on our website.

Dirk Englund:
We have studied the dependence of the NV center’s charge state and fluorescence on external potential variations for NDs with two different surface preparations: NDs with primarily hydroxyl surface termination groups (H-NDs) and NDs with primarily hydrogen surface termination groups (OH-NDs). We have shown that the NV fluorescence voltage response depends strongly on the ND surface termination, which affects both the underlying mechanism and the overall fraction of modulating NVs.
For OH-NDs, it is possible to observe 100 mV potential variations even with a single NV emitter, published in PNAS on April 2016 [5]. We have also evaluated the use of suspended artificial lipid bilayer membranes decorated with NV, which allows for the dis-entanglement of any electrical contact between NDs and the electrodes used to apply the voltage, and thus provides a better analogue of a live neuron membrane.

Sebastian Seung:

RPPR Final Report
as of 09-Feb-2022

We focused our work on connectomics on the problem of inference, the application of a previously trained ConvNet, with emphasis on 3D images. A CPU-GPU algorithm achieves the greatest throughput of all, 10x or more than other publicly available implementations of sliding window 3D ConvNets. All of our code has been made available as open source project.
Dense connectomic reconstructions offer the possibility of detailing connection between all neurons in a given volume. To do this in a selective way, we are developing a viral vector based tool that can label cells with both a fluorescent and electron dense reporter. In figure 1A-D, we show that that virus successfully expresses eFYP in cultured cells, and that is it highly Cre dependent. The infected cells then express DAB polymer (figure 1E) for EM, allowing the same cells to be identified by fluorescence microscopy and EM.

Jeff Lichtman:
1) We have published a reconstruction of a large region of mouse dorsal lateral geniculate nucleus (Morgan et al., 2016). This work was also previewed in a paper published by several other scientists in Cell (C. Chen, M. E. Bickford, & J. A. Hirsch “Untangling the Web between Eye and Brain” Cell, 2016 165:20-21).
In order to chart the parallel sensory streams passing through the visual thalamus, we acquired a 100-trillion- voxel electron microscopy dataset (i.e., 100TBs). This is the largest volume of brain ever studied in a connectomic way. We found a single large interconnected network that could not be easily subdivided, which is quite unlike the clear connectivity diagrams that are used to display putative canonical circuits and provides the first glimpse of the actual way the mammalian brain is organized.
2) We have written an in-depth review of the various ways circuits have been traced from Cajal to modern high and low resolution connectomic approaches (“From Cajal to Connectome and Beyond”, Swanson LW, Lichtman JW. Annu Rev Neurosci. 2016).

Xiaowei Zhang:
We previously demonstrated a large-volume super-resolution imaging platform that enables the reconstruction of neurons and synaptic fields in the nervous system by combining STORM imaging with serial ultrathin sectioning (Sigal et al., Cell 2015). We reconstructed an ‘Off-center’ direction-selective J-RGC and its inhibitory synaptic input field (Figure 1). We observed that synapse density is significantly higher near the soma and proximal dendrites and lower toward the periphery of the neuron.
In a separate project, we have now extended our study of synaptic organization to the mammalian neocortex. During development the brain undergoes epochs of increased sensitivity to sensory experience known as "critical periods", required for the establishment of mature neural circuits. Using our platform, we demonstrate that changes in the excitatory-inhibitory synaptic balance correlate with the observed shifts in the degree of maturation.

Jonathan Owen:
In this phase of the project, we have measured the voltage sensitivity of our spherical quantum well QDs and found it to be consistent with the voltage response predicted by theoretical descriptions of the quantum confined Stark effect (QCSE). In some cases, we observed ?F/F as large as 40% (E = 200 kV/cm) in thin film devices. In addition, a shift and broadening of the luminescence band were observed that are consistent with the QCSE simulations. In addition to finalizing the synthetic work and the voltage sensitivity measurements for publication, we have invested a significant amount of time testing the voltage response in model membranes with the Shepard group as well as neuronal staining using a liposome delivery strategy together with the Yuste lab.

Rafael Yuste:
We had three major accomplishments in the last funding cycle. The first one was to develop a new method to simultaneously image and photo-activate neurons in three dimensions, with two photon
excitation. The second accomplishment is to use this method to imprint neuronal ensembles into the brain of awake behaving mine. The third accomplishment is to develop methods to image voltage in vivo using genetically encoded voltage indicators. We plan to use this method to image the sub threshold activity of cortical ensembles.

Wei Min:
Simultaneous visualization of a large number of molecular species inside cells is essential in the era of systems biology. Herein we report a new imaging modality together with a matching reporter palette. Physically, we developed electronic pre-resonance stimulated Raman scattering (epr-SRS) microscopy. Chemically, we created a unique vibrational palette consisting of novel near-infrared dyes bearing conjugated and isotopically-edited triple bonds. Using this super-multiplex approach, we monitored DNA and protein metabolism in neuronal cultures and brain tissues. Here, we report our current progress in metabolic imaging of live rat brain hippocampal tissues in either physiological or pathological conditions, and the capture of active protein and lipid synthesis across the mouse brain tissue with single cell resolution.

Ozgur Sahin:
In order to directly measure electromechanical coupling in neurons we have developed a dual patch clamp and AFM approach. This novel approach allows for AFM measurements to be directly correlated with membrane voltage data for the same neuron. In addition, we can now directly change the membrane voltage and stimulate action potentials in the cell during our AFM measurements.
We have also combined AFM and optogenetic stimulation to study electromechanical coupling in different neuronal structures from the same neuron. While this approach does not provide a direct measurement of membrane voltage, it does allow reliable stimulation of action potentials without the physical obstructions to AFM scanning caused by the patch clamp. This method will allow us to study the mechanical and electrical properties of neurons in a novel way.

Ken Shepard:
65,536-channel multielectrode array. We have designed a 65,536-channel active CMOS multielectrode array that we are using to study the mouse retina. The array allows all 65,536 channels to be measured simultaneously, allowing 25-?m spatial resolution over a 64 mm2 area[1, 2].
Flexible CMOS wire

Training Opportunities: Personnel trained: 11 postdocs, 8 graduate students, 2 technicians

Results Dissemination: Publications: 45 peer-reviewed, several in top journals

Honors and Awards: Rafael Yuste
2016 8 Scientists Worldwide List, El Confidencial, Madrid
2017Congreso del Futuro, Senate of Chile, Santiago, Chile 2017Keynote Address, BiOS, Photonics West 2015, San Francisco, CA 2017Telva Science Prize, Valencia, Spain
2017Alumni Prize, Universidad Auto?noma de Madrid, Spain
2017Whitman Research Research Award Marine Biological Laboratory, Woods Hole, MA
2017Keynote Speaker, Centennial Symposium, Universidad de Cuenca, Ecuador 2017Keynote Speaker, Annual Prize Ceremony, Fundacion Cobos, Madrid, Spain 2017Dan Johnston Distinguished Lecture, UT-Austin, Texas
2020 Cajal Medal Diploma, Instituto Cajal, Madrid, Spain
2020 Vannevar Bush Faculty Award, Department of Defense, USA

Wei Min
2017 ACS Early Career Award of Experimental Physical Chemistry 2017 Coblentz Award of Molecular Spectroscopy

Elizabeth Hillman
Dr Hillman Elected Fellow of SPIE Dr Hillman Elected Fellow of AIMBE

Jeff Lichtman
Joseph Erlanger Distinguished Lectureship of the American Physiological Society
Edward J Masoro Distinguished Lectureship of the Department of Cellular & Integrative Physiology (UT San Antonio)
The Keynote lecture at 5 symposia (Manitoba, CSHL Asia, NIMH Postdoc Training Day, UT San Antonio Research day, Harvard leadership Summit)
NAS

Dirk Englund
The OSA's 2017 Adolph Lomb Medal
The 2017 ACS Photonics Young Investigator Award

Protocol Activity Status:

RPPR Final Report
as of 09-Feb-2022

Technology Transfer: Award # W911NF-12-1-0594

Names of Inventors Title of Invention Disclosure number,Patent Application Serial Number or Patent NumberElection to File Patent Application Confirmatory Instrument or Assignment Forwarded to Contracting Officer

			US		Foreign			
			Yes	No	Yes	No	Yes	No
1	Englund, Dirk	Deterministic Emitter Switch Microscopy (DESM)	1833202-11-0180; CU12035				x	
	Englund, Dirk	Conjugates Of Nano-Diamond And Magnetic Or Metallic Particles	14/150,412 (9,222,887)				x	
1a (These are listed on the iedison record, but this award was utilized only for the CIP filing - 14/150,412, so these will not indicate the W911NF-12-0594 funding)	Englund, Dirk	Deterministic Emitter Switch Microscopy (DESM)	61/549,046	x		x		
	Englund, Dirk	Deterministic Emitter Switch Microscopy (DESM)	61/562,551	x		x		
	Englund, Dirk	Deterministic Emitter Switch Microscopy (DESM)	61/591,570	x		x		
	Englund, Dirk	Deterministic Emitter Switch Microscopy (DESM)	61/624,647	x		x		
	Englund, Dirk	Systems and Methods for deterministic emitter switch mircosopy	PCT/US2012/060735	x	x			
	Englund, Dirk; Chen, Edward; Gaathon, Ophir	Systems And Methods For Deterministic Emitter Switch Microscopy	14/227,076 (9,632,045)	x		x		
	Englund, Dirk	Conjugates Of Nano-Diamond And Magnetic Or Metallic Particles	PCT/US2012/048830	x	x			
	Englund, Dirk	Conjugates Of Nano-Diamond And Magnetic Or Metallic Particles	14/952,216 (9,599,562)	x			x	
		Deterministic Emitter Switch Microscopy (DESM)	61/623,656			x		
	Englund, Dirk; Chen, Edward; Gaathon, Ophir	Systems and Methods for Deterministic Emitter Switch Microscopy (DESM)	61/805,776	x		x		
2	Shepard, Kenneth; Kang, Hongki; Tsai, David	Flexible complementary metal-oxide-semiconductor probes for chronic, large-scale neural stimulation and recording	1833202-14-0101; CU15023				x	
			62/210,532	x		x		
			PCT/US2016/049278		x		x	
	Shepard, Kenneth; Kang, Hongki; Tsai, David; Thimot, Jordan		15/906,206	x			x	
3	Shepard, Kenneth; Tsai, David	Methods and systems for dense, high-channel-count, multichannel data acquisition	1833202-16-0171; CU17180				x	
		Systems And Methods For Dense, High-Channel-Count, Multichannel Data Acquisition	62/486,741	x			x	
			62/492,580	x		x		
4	Shepard, Kenneth; Jayant, Krishna; Shekar, Siddarth	Miniaturized complementary metal-oxide-semiconductor (CMOS) multi-clamp amplifier	1833202-19-0016; CU19222				x	
		INTEGRATED ELECTROPHYSIOLOGY AMPLIFYING APPARATUS, COMPUTER-ACCESSIBLE MEDIUM, SYSTEM AND METHOD FOR USE THEREOF	62/852,587	x			x	
			PCT/US2020/034540		x		x	
5	Hillman, Elizabeth; Voleti, Venkatakaushik; Li, Wenzel; Patel, Kirpa; Campos, Citali; Bharadwaj, Srindhi; Casparr, Malte; Chentao, Wen; Targoff, Kimara; Ford, Caitlin; Kotaro, Kimura	SCAPE 2.0: A simple, reconfigurable microscope for ultra-high-speed, high-resolution, or large-scale volumetric imaging	1833202-19-0040; CU19278				x	
		Hihg Speed Microscopy Devices Methods and Systems	62/822,361	x			x	
6	Hillman, Elizabeth; Voleti, Venkatakaushik	SCAPE Microscopy with Phase Plate/Phase Modulating Device and Reconstructive Algorithm	1833202-16-0016; CU16255				x	
			62/343,112	x		x		
		SCAPE Microscopy with Phase Modulating Element and Image Reconstruction	PCT/US2017/034984	x	x			

16/304,755 x x

7 Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew Systems and Methods for Improved Laser Scanning Intersecting Plane Tomography 1833202-14-0006; CU14214 x
Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew Systems and Methods for Improved Laser Scanning Intersecting Plane Tomography 61/928,930 x x
Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew Scanning Oblique Light Sheet For Imaging High Speed Volumetric Microscopy 61/950,608 x x
Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew Systems And Methods For Three Dimensional Microscopy Imaging 61/955,482 x x
Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew; Mann, Richard; Mendes, Cesar; Bruno, Randy; Lacefield, Clay; Grueber, Wesley 61/969,712 x x
Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew 62/088,921 x x
Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew Systems And Methods For Three-Dimensional Imaging 62/126,278 x x
Hillman, Elizabeth; Bouchard, Matthew Systems And Methods For Improved Laser Scanning Intersecting Plane Tomography PCT/US2015/012076 x x
Hillman, Elizabeth Systems, Methods, And Devices For Imaging 62/195,729 x x
62/202,126 x x
Systems And Methods for Three Dimensional Imaging 15/211,128 (10,061,111) x x
16/042,700 x
Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew Systems and Methods for Three-Dimensional ImagingPCT/US2016/042398 x x
Hillman, Elizabeth Three-Dimensional Imaging Using Swept, Confocally Aligned Planar Excitation with an Image Relay 62/360,460 x x
PCT/US2017/041393 x x
Hillman, Elizabeth; Voleti, Venkatakaushik; Bouchard, Matthew 16/314,752 x x

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Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
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Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
National Academy Member: N

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Person Months Worked: 15.00 **Funding Support:**
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Journal: Nature Methods
Publication Identifier Type: DOI Publication Identifier: 10.1038/nmeth.2249
Volume: 9 Issue: 12 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Two-photon optogenetics of dendritic spines and neural circuits
Authors:
Keywords: Imaging, Mircroscopy, Neuroscience
Abstract: We demonstrate a two-photon optogenetic method that generates action potentials in neurons with single-cell precision, using the red-shifted opsin C1V1T. We applied the method to optically map synaptic circuits in mouse neocortical brain slices and to activate small dendritic regions and individual spines. Using a spatial light modulator, we split the laser beam onto several neurons and performed simultaneous optogenetic activation of selected neurons in three dimensions.
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Journal: ACS Chemical Neuroscience
Publication Identifier Type: DOI Publication Identifier: 10.1021/cn4000692
Volume: 0 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Two-photon optical interrogation of single dendritic spines with caged dopamine
Authors:
Keywords: Dopamine, rutheniu, two-photon, caged compound
Abstract: We introduce a novel caged dopamine compound (RuBi-Dopa) based on ruthenium photochemistry. RuBi-Dopa has a high uncaging efficiency and can be released with visible (blue-green) and IR light in a two-photon regime. We combine two-photon photorelease of RuBi-Dopa with two-photon calcium imaging for optical imaging and manipulation of dendritic spines in living brain slices, demonstrating that spines can express functional dopamine receptors. This novel compound allows mapping functional dopamine receptors in living brain tissue with exquisite spatial resolution.
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Journal:
Publication Identifier Type: DOI Publication Identifier: 10.1146/annurev-neuro-062111-150455
Volume: 36 Issue: 1 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Electrical Compartmentalization in Dendritic Spines
Authors:
Keywords: NMDA, computation, cortex, imaging, uncaging, network, emergent
Abstract: Most excitatory inputs in the CNS contact dendritic spines, avoiding dendritic shafts, so spines must play a key role for neurons. Recent data suggest that, in addition to enhancing connectivity and isolating synaptic biochemistry, spines can behave as electrical compartments independent from their parent dendrites. It is becoming clear that, al- though spines experience voltages similar to those of dendrites during action potentials (APs), spines must sustain higher depolarizations than do dendritic shafts during excitatory postsynaptic potentials (EPSPs). Synaptic potentials are likely amplified at the spine head and then re- duced as they invade the dendrite through the spine neck. These elec- trical changes, probably due to a combination of passive and active mechanisms, may prevent the saturation of dendrites by the joint acti- vation of many inputs, influence dendritic integration, and contribute to rapid synaptic plasticity. The electrical properties of spines could enable neura
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Journal: Optics Express
Publication Identifier Type: DOI Publication Identifier: 10.1364/OE.21.016007
Volume: 21 Issue: 13 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Instantaneous three-dimensional sensing using spatial light modulator illumination with extended depth of field imaging
Authors:
Keywords: Computational imaging; Functional monitoring and imaging; Three-dimensional microscopy
Abstract: Imaging three-dimensional structures represents a major challenge for conventional microscopies. Here we describe a Spatial Light Modulator (SLM) microscope that can simultaneously address and image multiple targets in three dimensions. A wavefront coding element and computational image processing enables extended depth-of-field imaging. High-resolution, multi-site three-dimensional targeting and sensing is demonstrated in both transparent and scattering media over a depth range of 300-1,000 microns.
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Journal: The Journal of Physical Chemistry Letters
Publication Identifier Type: DOI Publication Identifier: 10.1021/jz402128j
Volume: 0 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Bioluminescence Assisted Switching and Fluorescence Imaging (BASFI)
Authors:
Keywords: energy transfer; bioluminescence; photoswitching; protein?protein interaction; live-cell imaging
Abstract: Förster resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) are two major biophysical techniques for studying nanometer-scale motion dynamics within living cells. Both techniques read photoemission from the transient RET-excited acceptor, which makes RET and detection processes inseparable. We here report a novel hybrid strategy, bioluminescence assisted switching and fluorescence imaging (BASFI) using a bioluminescent Renilla luciferase RLuc8 as the donor and a photochromic fluorescent protein Dronpa as the acceptor. When in close proximity, RET from RLuc8 switches Dronpa from its original dark state to a stable bright state, whose fluorescence is imaged subsequently with an external laser. Such decoupling between RET and imaging processes in BASFI promises high photon flux as in FRET and minimal bleedthroughs as in BRET. We demonstrated BASFI with Dronpa-RLuc8 fusion constructs and drug-inducible intermolecular FKBP-FRB protein–protein interactions i
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Journal: Development
Publication Identifier Type: DOI Publication Identifier: 10.1242/dev.094631
Volume: 140 Issue: 13 First Page #: 2835
Date Submitted: Date Published:
Publication Location:
Article Title: Zebrabow: multispectral cell labeling for cell tracing and lineage analysis in zebrafish
Authors:
Keywords: Multispectral cell labeling, zebrafish
Abstract: Advances in imaging and cell-labeling techniques have greatly enhanced our understanding of developmental and neurobiological processes. Among vertebrates, zebrafish is uniquely suited for in vivo imaging owing to its small size and optical translucency. However, distinguishing and following cells over extended time periods remains difficult. Previous studies have demonstrated that Cre recombinase-mediated recombination can lead to combinatorial expression of spectrally distinct fluorescent proteins (RFP, YFP and CFP) in neighboring cells, creating a ‘Brainbow’ of colors. The random combination of fluorescent proteins provides a way to distinguish adjacent cells, visualize cellular interactions and perform lineage analyses. Here, we describe Zebrabow (Zebrafish Brainbow) tools for in vivo multicolor imaging in zebrafish. First, we show that the broadly expressed ubi:Zebrabow line provides diverse color profiles that can be optimized by modulating Cre activity. Second, we find that colo
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Journal: Nature Methods
Publication Identifier Type: DOI Publication Identifier: 10.1038/nmeth.2480
Volume: 10 Issue: 6 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Why not connectomics?
Authors:
Keywords: synaptic connectivity
Abstract: Opinions diverge on whether mapping the synaptic connectivity of the brain is a good idea. Here we argue that albeit their limitations, such maps will reveal essential characteristics of neural circuits that would otherwise be inaccessible.
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Journal: IEEE Transactions on Visualization and Computer Graphics
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Volume: 19 Issue: 12 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: ConnectomeExplorer: Query-Guided Visual Analysis of Large Volumetric Neuroscience Data
Authors:
Keywords: ConnectomeExplorer:
Abstract: This paper presents ConnectomeExplorer, an application for the interactive exploration and query-guided visual analysis of large volumetric electron microscopy (EM) data sets in connectomics research. Our system incorporates a knowledge-based query algebra that supports the interactive specification of dynamically evaluated queries, which enable neuroscientists to pose and answer domain-specific questions in an intuitive manner. Queries are built step by step in a visual query builder, building more complex queries from combinations of simpler queries. Our application is based on a scalable volume visualization framework that scales to multiple volumes of several teravoxels each, enabling the concurrent visualization and querying of the original EM volume, additional segmentation volumes, neuronal connectivity, and additional meta data comprising a variety of neuronal data attributes. We evaluate our application on a data set of roughly one terabyte of EM data and 750 GB of segmentatio
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: IEEE Computer Graphics and Applications
Publication Identifier Type: DOI Publication Identifier: 10.1109/MCG.2013.55
Volume: 33 Issue: 4 First Page #: 50
Date Submitted: Date Published:
Publication Location:
Article Title: Exploring the Connectome: Petascale Volume Visualization of Microscopy Data Streams
Authors:
Keywords: petascale data stream
Abstract: A system for interactively exploring petavoxel volumes from high-throughput electron microscopy data streams supports concurrent visualization of high- resolution volumes and voxel segmentation data. The visualization-driven system design handles incomplete data and improves scalability over previous approaches. Researchers have employed the system on a 1-teravoxel mouse cortex volume.
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Science
Publication Identifier Type: DOI Publication Identifier: 10.1126/science.1249766
Volume: 344 Issue: 6181 First Page #: 319
Date Submitted: Date Published:
Publication Location:
Article Title: Distinct Profiles of Myelin Distribution Along Single Axons of Pyramidal Neurons in the Neocortex
Authors:
Keywords: Myelin, aoxn, pyramidal neuron
Abstract: Myelin is a defining feature of the vertebrate nervous system. Variability in the thickness of the myelin envelope is a structural feature affecting the conduction of neuronal signals. Conversely, the distribution of myelinated tracts along the length of axons has been assumed to be uniform. Here, we traced high-throughput electron microscopy reconstructions of single axons of pyramidal neurons in the mouse neocortex and built high-resolution maps of myelination. We find that individual neurons have distinct longitudinal distribution of myelin. Neurons in the superficial layers displayed the most diversified profiles, including a new pattern where myelinated segments are interspersed with long, unmyelinated tracts. Our data indicate that the profile of longitudinal distribution of myelin is an integral feature of neuronal identity and may have evolved as a strategy to modulate long-distance communication in the neocortex.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Frontiers in Neural Circuits
Publication Identifier Type: DOI Publication Identifier: 10.3389/fncir.2014.00068
Volume: 8 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Imaging ATUM ultrathin section libraries with WaferMapper: a multi-scale approach to EM reconstruction of neural circuits
Authors:
Keywords: ATUM, WaferMapper
Abstract: The automated tape-collecting ultramicrotome (ATUM) makes it possible to collect large numbers of ultrathin sections quickly-the equivalent of a petabyte of high resolution images each day. However, even high throughput image acquisition strategies generate images far more slowly (at present ~1 terabyte per day). We therefore developed WaferMapper, a software package that takes a multi-resolution approach to mapping and imaging select regions within a library of ultrathin sections. This automated method selects and directs imaging of corresponding regions within each section of an ultrathin section library (UTSL) that may contain many thousands of sections. Using WaferMapper, it is possible to map thousands of tissue sections at low resolution and target multiple points of interest for high resolution imaging based on anatomical landmarks. The program can also be used to expand previously imaged regions, acquire data under different imaging conditions, or re-image after additional tiss
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Nature Methods
Publication Identifier Type: Publication Identifier:
Volume: 10 Issue: 6 First Page #: 540
Date Submitted: Date Published:
Publication Location:
Article Title: Improved tools for the Brainbow toolbox.
Authors:
Keywords: Brainbow
Abstract: In the transgenic multicolor labeling strategy called 'Brainbow', Cre-loxP recombination is used to create a stochastic choice of expression among fluorescent proteins, resulting in the indelible marking of mouse neurons with multiple distinct colors. This method has been adapted to non-neuronal cells in mice and to neurons in fish and flies, but its full potential has yet to be realized in the mouse brain. Here we present several lines of mice that overcome limitations of the initial lines, and we report an adaptation of the method for use in adeno-associated viral vectors. We also provide technical advice about how best to image Brainbow-expressing tissue.
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Journal of Physical Chemistry Letters
Publication Identifier Type: Publication Identifier:
Volume: 4 Issue: 0 First Page #: 3897
Date Submitted: Date Published:
Publication Location:
Article Title: Bioluminescence Assisted Switching and Fluorescence Imaging(BASFI)
Authors:
Keywords: Bioluminescence
Abstract: Forster resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) are two major biophysical techniques for studying nanometer- scale motion dynamics within living cells. Both techniques read photoemission from the transient RET-excited acceptor, which makes RET and detection processes inseparable. We here report a novel hybrid strategy, bioluminescence assisted switching and fluorescence imaging (BASFI) using a bioluminescent Renilla luciferase RLuc8 as the donor and a photochromic fluorescent protein Dronpa as the acceptor. When in close proximity, RET from RLuc8 switches Dronpa from its original dark state to a stable bright state, whose fluorescence is imaged subsequently with an external laser. Such decoupling between RET and imaging processes in BASFI promises high photon flux as in FRET and minimal bleedthroughs as in BRET. We demonstrated BASFI with Dronpa-RLuc8 fusion constructs and drug-inducible intermolecular FKBP-FRB protein?protein interactions
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Nature Methods
Publication Identifier Type: DOI Publication Identifier: 10.1038/nmeth.2878
Volume: 11 Issue: 4 First Page #: 410
Date Submitted: Date Published:
Publication Location:
Article Title: Live-cell imaging of alkyne-tagged small biomolecules by stimulated Raman scattering
Authors:
Keywords: Optical imaging Multiphoton microscopy
Abstract: Sensitive and specific visualization of small biomolecules in living systems is highly challenging. We report stimulated Raman-scattering imaging of alkyne tags as a general strategy for studying a broad spectrum of small biomolecules in live cells and animals. We demonstrate this technique by tracking alkyne-bearing drugs in mouse tissues and visualizing de novo synthesis of DNA, RNA, proteins, phospholipids and triglycerides through metabolic incorporation of alkyne-tagged small precursors.
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Journal: Frontiers of Neural Circuits
Publication Identifier Type: Publication Identifier:
Volume: 7 Issue: 0 First Page #: 1
Date Submitted: Date Published:
Publication Location:
Article Title: Classification of neocortical interneurons using affinitypropagation
Authors:
Keywords: affinity propagation; cell types; cortex; interneurons
Abstract: In spite of over a century of research on cortical circuits, it is still unknown how many classes of cortical neurons exist. In fact, neuronal classification is a difficult problem because it is unclear how to designate a neuronal cell class and what are the best characteristics to define them. Recently, unsupervised classifications using cluster analysis based on morphological, physiological, or molecular characteristics, have provided quantitative and unbiased identification of distinct neuronal subtypes, when applied to selected datasets. However, better and more robust classification methods are needed for increasingly complex and larger datasets. Here, we explored the use of affinity propagation, a recently developed unsupervised classification algorithm imported from machine learning, which gives a representative example or exemplar for each cluster. As a case study, we applied affinity propagation to a test dataset of 337 interneurons belonging to four subtypes, previously ident
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Current Opinion in Neurobiology
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.conb.2013.12.015
Volume: 26 Issue: 0 First Page #: 96
Date Submitted: Date Published:
Publication Location:
Article Title: A blanket of inhibition: functional inferences from dense inhibitory connectivity
Authors:
Keywords: interneurons cortex
Abstract: The function of neocortical interneurons is still unclear, and, as often happens, one may be able to draw functional insights from considering the structure. In this spirit we describe recent structural results and discuss their potential functional implications. Most GABAergic interneurons innervate nearby pyramidal neurons very densely and without any apparent specificity, as if they were extending a ‘blanket of inhibition’, contacting pyramidal neurons often in an overlapping fashion. While subtypes of interneurons specifically target subcellular compartments of pyramidal cells, and they also target different layers selectively, they appear to treat all neighboring pyramidal cells the same and innervate them massively. We explore the functional implications and temporal properties of dense, overlapping inhibition by four interneuron populations.
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Journal: Frontiers in Neural Circuits
Publication Identifier Type: DOI Publication Identifier: 10.3389/fncir.2014.00029
Volume: 8 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Simultaneous imaging of neural activity in three dimensions
Authors:
Keywords: brain activity map; calcium imaging; spatial-light-modulator; three-dimensional imaging; volume imaging
Abstract: We introduce a scanless optical method to image neuronal activity in three dimensions simultaneously. Using a spatial light modulator and a custom-designed phase mask, we illuminate and collect light simultaneously from different focal planes and perform calcium imaging of neuronal activity in vitro and in vivo. This method, combining structured illumination with volume projection imaging, could be used as a technological platform for brain activity mapping.
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Journal of the American Chemical Society
Publication Identifier Type: DOI Publication Identifier: 10.1021/ja5026326
Volume: 136 Issue: 24 First Page #: 8693
Date Submitted: Date Published:
Publication Location:
Article Title: Two-Photon Neuronal and Astrocytic Stimulation with Azobenzene-Based Photoswitches
Authors:
Keywords: Astrocyte, photoswitch
Abstract: Synthetic photochromic compounds can be designed to control a variety of proteins and their biochemical functions in living cells, but the high spatiotemporal precision and tissue penetration of two-photon stimulation have never been investigated in these molecules. Here we demonstrate two- photon excitation of azobenzene-based protein switches and versatile strategies to enhance their photochemical responses. This enables new applications to control the activation of neurons and astrocytes with cellular and subcellular resolution.
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Journal: Proceedings of the National Academy of Sciences
Publication Identifier Type: DOI Publication Identifier: 10.1073/pnas.1321869111
Volume: 111 Issue: 28 First Page #: 2895
Date Submitted: Date Published:
Publication Location:
Article Title: Activity-dependent dendritic spine neck changes are correlated with synaptic strength
Authors:
Keywords: STDP; basal dendrites; neocortex
Abstract: Most excitatory inputs in the mammalian brain are made on dendritic spines, rather than on dendritic shafts. Spines compartmentalize calcium, and this biochemical isolation can underlie input-specific synaptic plasticity, providing a raison d'etre for spines. However, recent results indicate that the spine can experience a membrane potential different from that in the parent dendrite, as though the spine neck electrically isolated the spine. Here we use two-photon calcium imaging of mouse neocortical pyramidal neurons to analyze the correlation between the morphologies of spines activated under minimal synaptic stimulation and the excitatory postsynaptic potentials they generate. We find that excitatory postsynaptic potential amplitudes are inversely correlated with spine neck lengths. Furthermore, a spike timing-dependent plasticity protocol, in which two-photon glutamate uncaging over a spine is paired with postsynaptic spikes, produces rapid shrinkage of the spine neck and concomita
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Nature Photonics
Publication Identifier Type: Publication Identifier:
Volume: 8 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Isotropic three-dimensional super-resolution imaging with a self-bending point spread function
Authors:
Keywords: 3D Super resolution
Abstract: Airy beams maintain their intensity profiles over a large propagation distance without substantial diffraction and exhibit lateral bending during propagation . This unique property has been exploited for the micromanipulation of particles , the generation of plasma channels and the guidance of plasmonic waves, but has not been explored for high-resolution optical microscopy. Here, we introduce a self-bending point spread function (SB-PSF) based on Airy beams for three- dimensional super-resolution fluorescence imaging. We designed a side-lobe-free SB-PSF and implemented a two-channel detection scheme to enable unambiguous three-dimensional localization of fluorescent molecules. The lack of diffraction and the propagation-dependent lateral bending make the SB-PSF well suited for precise three-dimensional localization of molecules over a large imaging depth. Using this method, we obtained super-resolution imaging with isotropic three-dimensional localization precision of 10–15 nm over a
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Journal: Journal of the American Chemical Society
Publication Identifier Type: DOI Publication Identifier: 10.1021/ja3105279
Volume: 135 Issue: 4 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Phosphine Quenching of Cyanine Dyes as a Versatile Tool for Fluorescence Microscopy
Authors:
Keywords: Fluorescence Microscopy
Abstract: We report that the cyanine dye Cy5 and several of its structural relatives are reversibly quenched by the phosphine TCEP (tris(2-carboxyethyl)phosphine). Using Cy5 as a model, we show that the quenching reaction occurs by 1,4-addition of the phosphine to the polymethine bridge of Cy5 to form a covalent adduct. Illumination with ultraviolet light dissociates the adduct and returns the dye to the fluorescent state. We demonstrate that TCEP quenching can be used for superresolution imaging as well as for other applications, such as differentiating between molecules inside and outside the cell.
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Journal: IEEE Journal of Solid-State Circuits
Publication Identifier Type: DOI Publication Identifier: 10.1109/JSSC.2013.2293777
Volume: 49 Issue: 4 First Page #: 867
Date Submitted: Date Published:
Publication Location:
Article Title: A 100 fps, Time-Correlated Single-Photon-Counting-Based Fluorescence-Lifetime Imager in 130 nm CMOS
Authors:
Keywords: SPAD Fluoresence lifetime
Abstract: A fully-integrated single-photon avalanche diode (SPAD) and time-to-digital converter (TDC) array for high-speed fluorescence lifetime imaging microscopy (FLIM) in standard 130 nm CMOS is presented. This imager is comprised of an array of 64-by-64 SPADs each with an independent TDC for performing time-correlated single-photon counting (TCSPC) at each pixel. The TDCs use a delay-locked-loop-based architecture and achieve a 62.5 ps resolution with up to a 64 ns range. A data-compression datapath is designed to transfer TDC data to off-chip buffers, which can support a data rate of up to 42 Gbps. These features, combined with a system implementation that leverages a x4 PCIe-cabled interface, allow for demonstrated FLIM imaging rates at up to 100 frames per second.
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Journal: Optics Express
Publication Identifier Type: DOI Publication Identifier: 10.1364/OE.21.028583
Volume: 21 Issue: 23 First Page #: 28583
Date Submitted: Date Published:
Publication Location:
Article Title: Fast compressed sensing analysis for super-resolution imaging using L1-homotopy
Authors:
Keywords: super-resolution
Abstract: In super-resolution imaging techniques based on single-molecule switching and localization, the time to acquire a super-resolution image is limited by the maximum density of fluorescent emitters that can be accurately localized per imaging frame. In order to increase the imaging rate, several methods have been recently developed to analyze images with higher emitter densities. One powerful approach uses methods based on compressed sensing to increase the analyzable emitter density per imaging frame by several-fold compared to other reported approaches. However, the computational cost of this approach, which uses interior point methods, is high, and analysis of a typical 40 μm x 40 μm field-of-view super-resolution movie requires thousands of hours on a high-end desktop personal computer. Here, we demonstrate an alternative compressed-sensing algorithm, L1-Homotopy (L1H), which can generate super-resolution image reconstructions that are essentially identical to those derived using inte
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Nature
Publication Identifier Type: DOI Publication Identifier: 10.1038/nature13240
Volume: 509 Issue: 7500 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Space–time wiring specificity supports direction selectivity in the retina
Authors:
Keywords: Amacrine Cells/cytology Amacrine Cells/physiology Amacrine Cells/ultrastructure Animals Artificial Intelligence Brain Mapping* Crowdsourcing Dendrites/metabolism Mice Models, Neurological* Motion Neural Pathways/physiology* Presynaptic Terminals/metabolism Retina/cytology* Retina/physiology* Retinal Bipolar Cells/cytology Retinal Bipolar Cells/physiology Retinal Bipolar Cells/ultrastructure Spatio-Temporal Analysis*
Abstract: How does the mammalian retina detect motion? This classic problem in visual neuroscience has remained unsolved for 50 years. In search of clues, here we reconstruct Off-type starburst amacrine cells (SACs) and bipolar cells (BCs) in serial electron microscopic images with help from EyeWire, an online community of 'citizen neuroscientists'. On the basis of quantitative analyses of contact area and branch depth in the retina, we find evidence that one BC type prefers to wire with a SAC dendrite near the SAC soma, whereas another BC type prefers to wire far from the soma. The near type is known to lag the far type in time of visual response. A mathematical model shows how such 'space-time wiring specificity' could endow SAC dendrites with receptive fields that are oriented in space-time and therefore respond selectively to stimuli that move in the outward direction from the soma.
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Journal: Nature Communications
Publication Identifier Type: DOI Publication Identifier: 10.1038/ncomms4512
Volume: 5 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: A genetic and computational approach to structurally classify neuronal types
Authors:
Keywords: genetic definition of cell types
Abstract: The importance of cell types in understanding brain function is widely appreciated but only a tiny fraction of neuronal diversity has been catalogued. Here we exploit recent progress in genetic definition of cell types in an objective structural approach to neuronal classification. The approach is based on highly accurate quantification of dendritic arbor position relative to neurites of other cells. We test the method on a population of 363 mouse retinal ganglion cells. For each cell, we determine the spatial distribution of the dendritic arbors, or arbor density, with reference to arbors of an abundant, well-defined interneuronal type. The arbor densities are sorted into a number of clusters that is set by comparison with several molecularly defined cell types. The algorithm reproduces the genetic classes that are pure types, and detects six newly clustered cell types that await genetic definition.
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Journal: Nature
Publication Identifier Type: DOI Publication Identifier: 10.1038/nature12346
Volume: 500 Issue: 7461 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Connectomic reconstruction of the inner plexiform layer in the mouse retina
Authors:
Keywords: Amacrine Cells/cytology Amacrine Cells/physiology Animals Cell Communication Connectome* Image Processing, Computer-Assisted Mice Mice, Inbred C57BL Microscopy, Electron Models, Biological* Neuropil/physiology Retina/cytology* Retina/physiology* Retinal Ganglion Cells/cytology Retinal Ganglion Cells/physiology*
Abstract: Comprehensive high-resolution structural maps are central to functional exploration and understanding in biology. For the nervous system, in which high resolution and large spatial extent are both needed, such maps are scarce as they challenge data acquisition and analysis capabilities. Here we present for the mouse inner plexiform layer--the main computational neuropil region in the mammalian retina--the dense reconstruction of 950 neurons and their mutual contacts. This was achieved by applying a combination of crowd-sourced manual annotation and machine-learning-based volume segmentation to serial block-face electron microscopy data. We characterize a new type of retinal bipolar interneuron and show that we can subdivide a known type based on connectivity. Circuit motifs that emerge from our data indicate a functional mechanism for a known cellular response in a ganglion cell that detects localized motion, and predict that another ganglion cell is motion sensitive. Amacrine Cells
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Journal: Nature Photonics
Publication Identifier Type: DOI Publication Identifier: 10.1038/nphoton.2014.323
Volume: 9.0E+000 Issue: 2.0E+000 First Page #: 113
Date Submitted: Date Published:
Publication Location:
Article Title: Swept confocally-aligned planar excitation (SCAPE) microscopy for high-speed volumetric imaging of behaving organisms
Authors:
Keywords: SCAPE , 3D microscopy technique, two-photon microscopy
Abstract: We report a new 3D microscopy technique that allows volumetric imaging of living samples at ultra-high speeds: Swept, confocally-aligned planar excitation (SCAPE) microscopy. While confocal and two-photon microscopy have revolutionized biomedical research, current implementations are costly, complex and limited in their ability to image 3D volumes at high speeds. Light-sheet microscopy techniques using two-objective, orthogonal illumination and detection require a highly constrained sample geometry, and either physical sample translation or complex synchronization of illumination and detection planes. In contrast, SCAPE microscopy acquires images using an angled, swept light-sheet in a single-objective, en-face geometry. Unique confocal descanning and image rotation optics map this moving plane onto a stationary high-speed camera, permitting completely translationless 3D imaging of intact samples at rates exceeding 20 volumes per second. We demonstrate SCAPE microscopy by ima
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Journal: Nature Methods
Publication Identifier Type: DOI Publication Identifier: 10.1038/nmeth.3292
Volume: 1.2E+001 Issue: 4.0E+000 First Page #: 319
Date Submitted: Date Published:
Publication Location:
Article Title: Ultrastructurally smooth thick partitioning and volume stitching for large-scale connectomics
Authors:
Keywords: Ultra Structure EM connectomics
Abstract: Focused-ion-beam scanning electron microscopy (FIB-SEM) has become an essential tool for studying neural tissue at resolutions below 10 nm × 10 nm × 10 nm, producing data sets optimized for automatic connectome tracing. We present a technical advance, ultrathick sectioning, which reliably subdivides embedded tissue samples into chunks (20 ?m thick) optimally sized and mounted for efficient, parallel FIB-SEM imaging. These chunks are imaged separately and then 'volume stitched' back together, producing a final three-dimensional data set suitable for connectome tracing.
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Publication Identifier Type: DOI Publication Identifier: 10.1016/j.media.2015.02.001
Volume: 2.2E+001 Issue: 1.0E+000 First Page #: 77
Date Submitted: Date Published:
Publication Location:
Article Title: Large-scale automatic reconstruction of neuronal processes from electron microscopy images
Authors:
Keywords: 3D reconstruction, Segmentation
Abstract: Automated sample preparation and electron microscopy enables acquisition of very large image data sets. These technical advances are of special importance to the field of neuroanatomy, as 3D reconstructions of neuronal processes at the nm scale can provide new insight into the fine grained structure of the brain. Segmentation of large-scale electron microscopy data is the main bottleneck in the analysis of these data sets. In this paper we present a pipeline that provides state-of-the art reconstruction performance while scaling to data sets in the GB-TB range. First, we train a random forest classifier on interactive sparse user annotations. The classifier output is combined with an anisotropic smoothing prior in a Conditional Random Field framework to generate multiple segmentation hypotheses per image. These segmentations are then combined into geometrically consistent 3D objects by segmentation fusion. We provide qualitative and quantitative evaluation of the automatic segmentation
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Journal: Nature Methods
Publication Identifier Type: DOI Publication Identifier: 10.1038/nmeth.3367
Volume: 1.2E+001 Issue: 6.0E+000 First Page #: 547
Date Submitted: Date Published:
Publication Location:
Article Title: Multispectral labeling technique to map many neighboring axonal projections in the same tissue
Authors:
Keywords: Neuronal Positional System (NPS), axonal arbors
Abstract: We describe a method to map the location of axonal arbors of many individual neurons simultaneously via the spectral properties of retrogradely transported dye-labeled vesicles. We inject overlapping regions of an axon target area with three or more different colored retrograde tracers. On the basis of the combinations and intensities of the colors in the individual vesicles transported to neuronal somata, we calculate the projection sites of each neuron's axon. This neuronal positioning system (NPS) enables mapping of many axons in a simple automated way. In our experiments, NPS combined with spectral (Brainbow) labeling of the input to autonomic ganglion cells showed that the locations of ganglion cell projections to a mouse salivary gland related to the identities of their preganglionic axonal innervation. NPS could also delineate projections of many axons simultaneously in the mouse central nervous system.
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Journal: Cell
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.cell.2015.06.067
Volume: 1.62E+002 Issue: 2.0E+000 First Page #: 246
Date Submitted: Date Published:
Publication Location:
Article Title: Clarifying Tissue Clearing
Authors:
Keywords: Deep Tissue Imaging
Abstract: Biological specimens are intrinsically three dimensional; however, because of the obscuring effects of light scatter, imaging deep into a tissue volume is problematic. Although efforts to eliminate the scatter by "clearing" the tissue have been ongoing for over a century, there have been a large number of recent innovations. This Review introduces the physical basis for light scatter in tissue, describes the mechanisms underlying various clearing techniques, and discusses several of the major advances in light microscopy for imaging cleared tissue.
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Journal: Cell
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.cell.2015.06.054
Volume: 1.62E+002 Issue: 3.0E+000 First Page #: 648
Date Submitted: Date Published:
Publication Location:
Article Title: Saturated Reconstruction of a Volume of Neocortex
Authors:
Keywords: saturated reconstruction, Peters' rule
Abstract: We describe automated technologies to probe the structure of neural tissue at nanometer resolution and use them to generate a saturated reconstruction of a sub-volume of mouse neocortex in which all cellular objects (axons, dendrites, and glia) and many sub-cellular components (synapses, synaptic vesicles, spines, spine apparati, postsynaptic densities, and mitochondria) are rendered and itemized in a database. We explore these data to study physical properties of brain tissue. For example, by tracing the trajectories of all excitatory axons and noting their juxtapositions, both synaptic and non-synaptic, with every dendritic spine we refute the idea that physical proximity is sufficient to predict synaptic connectivity (the so-called Peters' rule). This online minable database provides general access to the intrinsic complexity of the neocortex and enables further data-driven inquiries.
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Journal: The Journal of Physical Chemistry C
Publication Identifier Type: DOI Publication Identifier: 10.1021/jp506992c
Volume: 1.18E+002 Issue: 4.6E+001 First Page #: 26695
Date Submitted: Date Published:
Publication Location:
Article Title: Surface Structure of Aerobically Oxidized Diamond Nanocrystals
Authors:
Keywords: diamond nanocrystals,
Abstract: We investigate the aerobic oxidation of high-pressure, high-temperature nanodiamonds (5-50 nm dimensions) using a combination of carbon and oxygen K-edge X-ray absorption, wavelength-dependent X-ray photoelectron, and vibrational spectroscopies. Oxidation at 575 °C for 2 h eliminates graphitic carbon contamination (>98%) and produces nanocrystals with hydroxyl functionalized surfaces as well as a minor component (<5%) of carboxylic anhydrides. The low graphitic carbon content and the high crystallinity of HPHT are evident from Raman spectra acquired using visible wavelength excitation (?excit = 633 nm) as well as carbon K-edge X-ray absorption spectra where the signature of a core-hole exciton is observed. Both spectroscopic features are similar to those of chemical vapor deposited (CVD) diamond but differ significantly from the spectra of detonation nanodiamond. The importance of these findings to the functionalization of nanodiamond surfaces for biological labeling applications is di
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: IEEE Transactions on Circuits and Systems I: Regular Papers
Publication Identifier Type: DOI Publication Identifier: 10.1109/TCSI.2015.2426958
Volume: 6.2E+001 Issue: 8.0E+000 First Page #: 1950
Date Submitted: Date Published:
Publication Location:
Article Title: Matching the Power, Voltage, and Size of Biological Systems: A nW-Scale, 0.023-<formula formulatype="inline"> <tex Notation="TeX">${\rm mm}^{3}$</tex></formula> Pulsed 33-GHz Radio Transmitter Operating From a 5 kT/q-Supply Voltage
Authors:
Keywords: Antennas, low power design, monolithic inte-gratedcircuits,radio frequency oscillators
Abstract: This paper explores the extent to which a solid-state transmitter can be miniaturized, while still using RF for wireless information transfer and working with power densities and operating voltages comparable to what could be harvested from a living system. A 3.1 nJ/bit pulsed millimeter-wave transmitter, 300 by 300 by 250 in size, designed in 32-nm SOI CMOS, operates on an electric potential of 130 mV and 3.1 nW of dc power. Farfield data transmission at 33 GHz is achieved by supply-switching an LC-oscillator with a duty cycle of . The time interval between pulses carries information on the amount of power harvested by the radio, supporting a data rate of 1 bps. The inductor of the oscillator also acts as an electrically small on-chip antenna, which, combined with millimeter-wave operation, enables the extremely small form factor.
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Nature Neuroscience
Publication Identifier Type: DOI Publication Identifier: 10.1038/nn.3720
Volume: 1.7E+001 Issue: 6.0E+000 First Page #: 866
Date Submitted: Date Published:
Publication Location:
Article Title: Spatiotemporal receptive fields of barrel cortex revealed by reverse correlation of synaptic input
Authors:
Keywords: Barrel cortex
Abstract: Of all of the sensory areas, barrel cortex is among the best understood in terms of circuitry, yet least understood in terms of sensory function. We combined intracellular recording in rats with a multi-directional, multi-whisker stimulator system to estimate receptive fields by reverse correlation of stimuli to synaptic inputs. Spatiotemporal receptive fields were identified orders of magnitude faster than by conventional spike-based approaches, even for neurons with little spiking activity. Given a suitable stimulus representation, a linear model captured the stimulus-response relationship for all neurons with high accuracy. In contrast with conventional single-whisker stimuli, complex stimuli revealed markedly sharpened receptive fields, largely as a result of adaptation. This phenomenon allowed the surround to facilitate rather than to suppress responses to the principal whisker. Optimized stimuli enhanced firing in layers 4-6, but not in layers 2/3, which remained sparsely active.
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: PLoS Computational Biology
Publication Identifier Type: DOI Publication Identifier: 10.1371/journal.pcbi.1004288
Volume: 1.1E+001 Issue: 6.0E+000 First Page #: 11
Date Submitted: Date Published:
Publication Location:
Article Title: Encoder-Decoder Optimization for Brain-Computer Interfaces
Authors:
Keywords: Brain-computer interfaces
Abstract: Neuroprosthetic brain-computer interfaces are systems that decode neural activity into useful control signals for effectors, such as a cursor on a computer screen. It has long been recognized that both the user and decoding system can adapt to increase the accuracy of the end effector. Co-adaptation is the process whereby a user learns to control the system in conjunction with the decoder adapting to learn the user's neural patterns. We provide a mathematical framework for co-adaptation and relate co-adaptation to the joint optimization of the user's control scheme ("encoding model") and the decoding algorithm's parameters. When the assumptions of that framework are respected, co-adaptation cannot yield better performance than that obtainable by an optimal initial choice of fixed decoder, coupled with optimal user learning. For a specific case, we provide numerical methods to obtain such an optimized decoder. We demonstrate our approach in a model brain-computer interface system using
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Journal: Cell
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.cell.2015.06.036
Volume: 1.62E+002 Issue: 2.0E+000 First Page #: 338
Date Submitted: Date Published:
Publication Location:
Article Title: Primacy of Flexor Locomotor Pattern Revealed by Ancestral Reversion of Motor Neuron Identity
Authors:
Keywords: motor neuron, skies
Abstract: Spinal circuits can generate locomotor output in the absence of sensory or descending input, but the principles of locomotor circuit organization remain unclear. We sought insight into these principles by considering the elaboration of locomotor circuits across evolution. The identity of limb-innervating motor neurons was reverted to a state resembling that of motor neurons that direct undulatory swimming in primitive aquatic vertebrates, permitting assessment of the role of motor neuron identity in determining locomotor pattern. Two-photon imaging was coupled with spike inference to measure locomotor firing in hundreds of motor neurons in isolated mouse spinal cords. In wild-type preparations, we observed sequential recruitment of motor neurons innervating flexor muscles controlling progressively more distal joints. Strikingly, after reversion of motor neuron identity, virtually all firing patterns became distinctly flexor like. Our findings show that motor neuron identity directs loc
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Frontiers in Neuroanatomy
Publication Identifier Type: DOI Publication Identifier: 10.3389/fnana.2014.00139
Volume: 8.0E+000 Issue: 0 First Page #: 7
Date Submitted: Date Published:
Publication Location:
Article Title: Automated computation of arbor densities: a step toward identifying neuronal cell types
Authors:
Keywords: cell types, classification, retinal ganglion cells, reconstruction, stratification, laminar structures
Abstract: The shape and position of a neuron convey information regarding its molecular and functional identity. The identification of cell types from structure, a classic method, relies on the time-consuming step of arbor tracing. However, as genetic tools and imaging methods make data-driven approaches to neuronal circuit analysis feasible, the need for automated processing increases. Here, we first establish that mouse retinal ganglion cell types can be as precise about distributing their arbor volumes across the inner plexiform layer as they are about distributing the skeletons of the arbors. Then, we describe an automated approach to computing the spatial distribution of the dendritic arbors, or arbor density, with respect to a global depth coordinate based on this observation. Our method involves three-dimensional reconstruction of neuronal arbors by a supervised machine learning algorithm, post-processing of the enhanced stacks to remove somata and isolate the neuron of interest, and regi
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Journal: ACS Chemical Biology
Publication Identifier Type: DOI Publication Identifier: 10.1021/cb500787b
Volume: 1.0E+001 Issue: 3.0E+000 First Page #: 901
Date Submitted: Date Published:
Publication Location:
Article Title: Imaging Complex Protein Metabolism in Live Organisms by Stimulated Raman Scattering Microscopy with Isotope Labeling
Authors:
Keywords: Protein, metabolism, stimulated Raman scattering (SRS) microscopy
Abstract: Protein metabolism, consisting of both synthesis and degradation, is highly complex, playing an indispensable regulatory role throughout physiological and pathological processes. Over recent decades, extensive efforts, using approaches such as autoradiography, mass spectrometry, and fluorescence microscopy, have been devoted to the study of protein metabolism. However, noninvasive and global visualization of protein metabolism has proven to be highly challenging, especially in live systems. Recently, stimulated Raman scattering (SRS) microscopy coupled with metabolic labeling of deuterated amino acids (D-AAs) was demonstrated for use in imaging newly synthesized proteins in cultured cell lines. Herein, we significantly generalize this notion to develop a comprehensive labeling and imaging platform for live visualization of complex protein metabolism, including synthesis, degradation, and pulse-chase analysis of two temporally defined populations. First, the deuterium labeling efficienc

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Publication Identifier Type: DOI Publication Identifier: 10.1002/anie.201502543
Volume: 5.4E+001 Issue: 3.4E+001 First Page #: 9821
Date Submitted: Date Published:
Publication Location:
Article Title: Vibrational Imaging of Glucose Uptake Activity in Live Cells and Tissues by Stimulated Raman Scattering
Authors:
Keywords: Raman spectroscopy; SRS microscopy; alkyne tags; glucose uptake; imaging agents
Abstract: Glucose is a ubiquitous energy source for most living organisms. Its uptake activity closely reflects cellular metabolic demand in various physiopathological conditions. Extensive efforts have been made to specifically image glucose uptake, such as with positron emission tomography, magnetic resonance imaging, and fluorescence microscopy, but all have limitations. A new platform to visualize glucose uptake activity in live cells and tissues is presented that involves performing stimulated Raman scattering on a novel glucose analogue labeled with a small alkyne moiety. Cancer cells with differing metabolic activities can be distinguished. Heterogeneous uptake patterns are observed with clear cell-cell variations in tumor xenograft tissues, neuronal culture, and mouse brain tissues. By offering the distinct advantage of optical resolution but without the undesirable influence of fluorophores, this method will facilitate the study of energy demands of living systems with subcellular resol

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Journal: Cell
Publication Identifier Type: Publication Identifier:
Volume: 0 Issue: 0 First Page #: 0
Date Submitted: Date Published:
Publication Location:
Article Title: Mapping synaptic input fields of neurons with super-resolution imaging.
Authors:
Keywords: synaptic input fields, On-Off direction-selective ganglion cells (On-Off DSGCs)
Abstract: As a basic functional unit in neural circuits, each neuron integrates input signals from hundreds to thousands of synapses. Knowledge of the synaptic input fields of individual neurons, including the identity, strength and location of each synapse, is essential for understanding how neurons compute. Here we developed a volumetric super-resolution reconstruction platform for large-volume imaging and automated segmentation of neurons and synapses with molecular identity information. We used this platform to map inhibitory synaptic input fields of On-Off direction-selective ganglion cells (On-Off DSGCs), which are important for computing visual motion direction in the mouse retina. The reconstructions of On-Off DSGCs showed a GABAergic, receptor subtype-specific input field for generating direction selective responses without significant glycinergic inputs for mediating monosynaptic crossover inhibition. These results demonstrate unique capabilities of this super-resoluti
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Journal: Nature Reveiws Neuroscience
Publication Identifier Type: DOI Publication Identifier: 10.1038/nrn3962
Volume: 1.6E+001 Issue: 8.0E+000 First Page #: 487
Date Submitted: Date Published:
Publication Location:
Article Title: From the neuron doctrine to neural networks
Authors:
Keywords: Attractors, Grid cells, learning rule, Emsembles, pattern completion, pereptrons, replay
Abstract: For over a century, the neuron doctrine - which states that the neuron is the structural and functional unit of the nervous system - has provided a conceptual foundation for neuroscience. This viewpoint reflects its origins in a time when the use of single-neuron anatomical and physiological techniques was prominent. However, newer multineuronal recording methods have revealed that ensembles of neurons, rather than individual cells, can form physiological units and generate emergent functional properties and states. As a new paradigm for neuroscience, neural network models have the potential to incorporate knowledge acquired with single-neuron approaches to help us understand how emergent functional states generate behaviour, cognition and mental disease.
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Journal: Journal of Neuroscience
Publication Identifier Type: DOI Publication Identifier: 10.1523/JNEUROSCI.5214-14.2015
Volume: 3.5E+001 Issue: 2.3E+001 First Page #: 8813
Date Submitted: Date Published:
Publication Location:
Article Title: Endogenous Sequential Cortical Activity Evoked by Visual Stimuli
Authors:
Keywords: graph theory; in vivo calcium imaging; multidimensional population vectors; neuronal ensembles; primary visual cortex; two-photon microscopy
Abstract: Although the functional properties of individual neurons in primary visual cortex have been studied intensely, little is known about how neuronal groups could encode changing visual stimuli using temporal activity patterns. To explore this, we used in vivo two-photon calcium imaging to record the activity of neuronal populations in primary visual cortex of awake mice in the presence and absence of visual stimulation. Multidimensional analysis of the network activity allowed us to identify neuronal ensembles defined as groups of cells firing in synchrony. These synchronous groups of neurons were themselves activated in sequential temporal patterns, which repeated at much higher proportions than chance and were triggered by specific visual stimuli such as natural visual scenes. Interestingly, sequential patterns were also present in recordings of spontaneous activity without any sensory stimulation and were accompanied by precise firing sequences at the single-cell level. Moreover, intri

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Journal: Frontiers in Neuroanatomy
Publication Identifier Type: DOI Publication Identifier: 10.3389/fnana.2015.00018
Volume: 9.0E+000 Issue: 0 First Page #: 18
Date Submitted: Date Published:
Publication Location:
Article Title: The discovery of dendritic spines by Cajal
Authors:
Keywords: Cajal; Golgi; cerebellum; cortex; dendritic spines
Abstract: Dendritic spines were considered an artifact of the Golgi method until a brash Spanish histologist, Santiago Ramón y Cajal, bet his scientific career arguing that they were indeed real, correctly deducing their key role in mediating synaptic connectivity. This article reviews the historical context of the discovery of spines and the reasons behind Cajal's obsession with them, all the way till his deathbed.

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Publication Identifier Type: DOI Publication Identifier: 10.3389/fnins.2015.00025
Volume: 9.0E+000 Issue: 0 First Page #: 25
Date Submitted: Date Published:
Publication Location:
Article Title: Optical triggered seizures using a caged 4-Aminopyridine
Authors:
Keywords: caged compound; electrophysiology; epilepsy model; neocortex; optical imaging; photostimulation
Abstract: Animal models of epilepsy are critical not only for understanding the fundamental mechanism of epilepsy but also for testing the efficacy of new antiepileptic drugs and novel therapeutic interventions. Photorelease of caged molecules is widely used in biological research to control pharmacologic events with high spatio-temporal resolution. We developed a technique for in vivo optical triggering of neocortical seizures using a novel caged compound based on ruthenium photochemistry (RuBi-4AP). Epileptiform events in mouse cortex were induced with blue light in both whole brain and focal illumination. Multi-electrode array recording and optical techniques were used to characterize the propagation of these epileptic events, including interictal spikes, polyspikes, and ictal discharges. These results demonstrate a novel optically-triggered seizure model, with high spatio-temporal control, that could have widespread application in the investigation of ictal onset, propagation and to develop
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Journal: Journal of Neuroscience
Publication Identifier Type: DOI Publication Identifier: 10.1523/JNEUROSCI.1085-14.2014
Volume: 3.4E+001 Issue: 3.0E+001 First Page #: 10078
Date Submitted: Date Published:
Publication Location:
Article Title: Random Positions of Dendritic Spines in Human Cerebral Cortex
Authors:
Keywords: Fourier; Lucifer; intracellular injections; pyramidal cells; spatial distribution
Abstract: Dendritic spines establish most excitatory synapses in the brain and are located in Purkinje cell's dendrites along helical paths, perhaps maximizing the probability to contact different axons. To test whether spine helixes also occur in neocortex, we reconstructed >500 dendritic segments from adult human cortex obtained from autopsies. With Fourier analysis and spatial statistics, we analyzed spine position along apical and basal dendrites of layer 3 pyramidal neurons from frontal, temporal, and cingulate cortex. Although we occasionally detected helical positioning, for the great majority of dendrites we could not reject the null hypothesis of spatial randomness in spine locations, either in apical or basal dendrites, in neurons of different cortical areas or among spines of different volumes and lengths. We conclude that in adult human neocortex spine positions are mostly random. We discuss the relevance of these results for spine formation and plasticity and their functional impact
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Journal: Brain Structure and Function
Publication Identifier Type: DOI Publication Identifier: 10.1007/s00429-014-0828-3
Volume: 2.2E+002 Issue: 5.0E+000 First Page #: 2817
Date Submitted: Date Published:
Publication Location:
Article Title: Spatial distribution of neurons innervated by chandelier cells
Authors:
Keywords: Chandelier, axo-axonic, GABAergic interneurons, biocytin reconstruction
Abstract: Chandelier (or axo-axonic) cells are a distinct group of GABAergic interneurons that innervate the axon initial segments of pyramidal cells and are thus thought to have an important role in controlling the activity of cortical circuits. To examine the circuit connectivity of chandelier cells (ChCs), we made use of a genetic targeting strategy to label neocortical ChCs in upper layers of juvenile mouse neocortex. We filled individual ChCs with biocytin in living brain slices and reconstructed their axonal arbors from serial semi-thin sections. We also reconstructed the cell somata of pyramidal neurons that were located inside the ChC axonal trees and determined the percentage of pyramidal neurons whose axon initial segments were innervated by ChC terminals. We found that the total percentage of pyramidal neurons that were innervated by a single labeled ChC was 18-22 %. Sholl analysis showed that this percentage peaked at 22-35 % for distances between 30 and 60 µm from the ChC soma, decr
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Proceedings of the National Academy of Sciences
Publication Identifier Type: DOI Publication Identifier: 10.1073/pnas.1406077111
Volume: 1.11E+002 Issue: 3.8E+001 First Page #: 38
Date Submitted: Date Published:
Publication Location:
Article Title: Visual stimuli recruit intrinsically generated cortical ensembles
Authors:
Keywords: V1; assemblies; mouse; reverberation
Abstract: The cortical microcircuit is built with recurrent excitatory connections, and it has long been suggested that the purpose of this design is to enable intrinsically driven reverberating activity. To understand the dynamics of neocortical intrinsic activity better, we performed two-photon calcium imaging of populations of neurons from the primary visual cortex of awake mice during visual stimulation and spontaneous activity. In both conditions, cortical activity is dominated by coactive groups of neurons, forming ensembles whose activation cannot be explained by the independent firing properties of their contributing neurons, considered in isolation. Moreover, individual neurons flexibly join multiple ensembles, vastly expanding the encoding potential of the circuit. Intriguingly, the same coactive ensembles can repeat spontaneously and in response to visual stimuli, indicating that stimulus-evoked responses arise from activating these intrinsic building blocks. Although the spatial prop
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Journal: Current Biology
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.cub.2017.06.028
Volume: 27 Issue: 14 First Page #: 2137
Date Submitted: 9/7/17 12:00AM Date Published: 7/1/17 4:00AM
Publication Location:
Article Title: Electron Microscopic Reconstruction of Functionally Identified Cells in a Neural Integrator
Authors: Ashwin Vishwanathan, Kayvon Daie, Alexandro D. Ramirez, Jeff W. Lichtman, Emre R.F. Aksay, H. Sek
Keywords: connectomics; eye-position; neural integrator; positive feedback; serial section electron microscopy; two-photon microscopy; zebrafish
Abstract: Neural integrators are involved in a variety of sensorimotor and cognitive behaviors. The oculomotor system contains a simple example, a hindbrain neural circuit that takes velocity signals as inputs and temporally integrates them to control eye position. Here we investigated the structural underpinnings of temporal integration in the larval zebrafish by first identifying integrator neurons using two-photon calcium imaging and then reconstructing the same neurons through serial electron microscopic analysis. Integrator neurons were identified as those neurons with activities highly correlated with eye position during spontaneous eye movements. Three morphological classes of neurons were observed: ipsilaterally projecting neurons located medially, contralaterally projecting neurons located more laterally, and a population at the extreme lateral edge of the hindbrain for which we were not able to identify axons. Based on their somatic locations, we inferred that neurons with only ipsilat
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Journal: Scientific Reports
Publication Identifier Type: DOI Publication Identifier: 10.1038/srep39660
Volume: 6 Issue: 1 First Page #:
Date Submitted: 9/7/17 12:00AM Date Published: 12/1/16 5:00AM
Publication Location:
Article Title: Bioorthogonal chemical imaging of metabolic activities in live mammalian hippocampal tissues with stimulated Raman scattering
Authors: Fanghao Hu, Michael R. Lamprecht, Lu Wei, Barclay Morrison, Wei Min
Keywords: Biophysical chemistry, Multiphoton microscopy
Abstract: Brain is an immensely complex system displaying dynamic and heterogeneous metabolic activities. Visualizing cellular metabolism of nucleic acids, proteins, and lipids in brain with chemical specificity has been a long-standing challenge. Recent development in metabolic labeling of small biomolecules allows the study of these metabolisms at the global level. However, these techniques generally require nonphysiological sample preparation for either destructive mass spectrometry imaging or secondary labeling with relatively bulky fluorescent labels. In this study, we have demonstrated bioorthogonal chemical imaging of DNA, RNA, protein and lipid metabolism in live rat brain hippocampal tissues by coupling stimulated Raman scattering microscopy with integrated deuterium and alkyne labeling. Heterogeneous metabolic incorporations for different molecular species and neurogenesis with newly-incorporated DNA were observed in the dentate gyrus of hippocampus at the single cell level. We further
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Volume: 544 Issue: 7651 First Page #: 465
Date Submitted: 9/7/17 12:00AM Date Published: 4/1/17 4:00AM
Publication Location:
Article Title: Super-multiplex vibrational imaging
Authors: Lu Wei, Zhixing Chen, Lixue Shi, Rong Long, Andrew V. Anzalone, Luyuan Zhang, Fanghao Hu, Rafael
Keywords: Raman spectroscopy, Multiphoton microscopy, Chemical biology, Biophysical chemistry
Abstract: The ability to visualize directly a large number of distinct molecular species inside cells is increasingly essential for understanding complex systems and processes. Even though existing methods have successfully been used to explore structure-function relationships in nervous systems, to profile RNA in situ, to reveal the heterogeneity of tumour microenvironments and to study dynamic macromolecular assembly, it remains challenging to image many species with high selectivity and sensitivity under biological conditions. For instance, fluorescence microscopy faces a 'colour barrier', owing to the intrinsically broad (about 1,500 inverse centimetres) and featureless nature of fluorescence spectra that limits the number of resolvable colours to two to five (or seven to nine if using complicated instrumentation and analysis). Spontaneous Raman microscopy probes vibrational transitions with much narrower resonances (peak width of about 10 inverse centimetres) and so does not suffer from thi
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Journal: Chem. Commun.
Publication Identifier Type: DOI Publication Identifier: 10.1039/C7CC01860F
Volume: 53 Issue: 46 First Page #: 6187
Date Submitted: 9/7/17 12:00AM Date Published:
Publication Location:
Article Title: Stimulated Raman scattering of polymer nanoparticles for multiplexed live-cell imaging
Authors: Fanghao Hu, Spencer D. Brucks, Tristan H. Lambert, Luis M. Campos, Wei Min
Keywords: Raman microscopy
Abstract: A novel nanoparticle-based imaging strategy is introduced that couples biocompatible organic nanoparticles and stimulated Raman scattering (SRS) microscopy. Polymer nanoparticles with vibrational labels incorporated were readily prepared for multi-color SRS imaging with excellent photo-stability. The Raman-active polymer dots are nontoxic, rapidly enter various cell types, and are applied in multiplexed cell-type sorting.
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Publication Identifier Type: DOI Publication Identifier: 10.1039/C7AN01001J
Volume: Issue: First Page #:
Date Submitted: 9/7/17 12:00AM Date Published:
Publication Location:
Article Title: Applications of vibrational tags in biological imaging by Raman microscopy
Authors: Zhilun Zhao, Yihui Shen, Fanghao Hu, Wei Min
Keywords: Raman microscopy
Abstract: As a superb tool to visualize and study the spatial-temporal distribution of chemicals, Raman microscopy has made a big impact in many disciplines of science. While label-free imaging has been the prevailing strategy in Raman microscopy, recent development and applications of vibrational/Raman tags, particularly when coupled with stimulated Raman scattering (SRS) microscopy, have generated intense excitement in biomedical imaging. SRS imaging of vibrational tags has enabled researchers to study a wide range of small biomolecules with high specificity, sensitivity and multiplex capability, at a single live cell level, tissue level or even in vivo. As reviewed in this article, this platform has facilitated imaging distribution and dynamics of small molecules such as glucose, lipids, amino acids, nucleic acids, and drugs that are otherwise difficult to monitor with other means. As both the vibrational tags and Raman instrumental development progress rapidly and synergistically, we anticip
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Journal: Neuron
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.neuron.2015.12.012
Volume: 89 Issue: 2 First Page #: 269
Date Submitted: 9/7/17 12:00AM Date Published: 1/1/16 5:00AM
Publication Location:
Article Title: Simultaneous Multi-plane Imaging of Neural Circuits
Authors: Weijian Yang, Jae-eun Kang Miller, Luis Carrillo-Reid, Eftychios Pnevmatikakis, Liam Paninski, Rafael Y
Keywords: SLM, two-photon microscopy
Abstract: Recording the activity of large populations of neurons is an important step toward understanding the emergent function of neural circuits. Here we present a simple holographic method to simultaneously perform two-photon calcium imaging of neuronal populations across multiple areas and layers of mouse cortex in vivo. We use prior knowledge of neuronal locations, activity sparsity, and a constrained nonnegative matrix factorization algorithm to extract signals from neurons imaged simultaneously and located in different focal planes or fields of view. Our laser multiplexing approach is simple and fast, and could be used as a general method to image the activity of neural circuits in three dimensions across multiple areas in the brain.
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Volume: 89 Issue: 2 First Page #: 285
Date Submitted: 9/7/17 12:00AM Date Published: 1/1/16 5:00AM
Publication Location:
Article Title: Simultaneous Denoising, Deconvolution, and Demixing of Calcium Imaging Data
Authors: Eftychios A. Pnevmatikakis, Daniel Soudry, Yuanjun Gao, Timothy A. Machado, Josh Merel, David Pfau
Keywords: calcium imaging data analysis
Abstract: We present a modular approach for analyzing calcium imaging recordings of large neuronal ensembles. Our goal is to simultaneously identify the locations of the neurons, demix spatially overlapping components, and denoise and deconvolve the spiking activity from the slow dynamics of the calcium indicator. Our approach relies on a constrained nonnegative matrix factorization that expresses the spatiotemporal fluorescence activity as the product of a spatial matrix that encodes the spatial footprint of each neuron in the optical field and a temporal matrix that characterizes the calcium concentration of each neuron over time. This framework is combined with a novel constrained deconvolution approach that extracts estimates of neural activity from fluorescence traces, to create a spatiotemporal processing algorithm that requires minimal parameter tuning. We demonstrate the general applicability of our method by applying it to in vitro and in vivo multi-neuronal imaging data, whole-brain li
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Journal: Frontiers in Neuroinformatics
Publication Identifier Type: DOI Publication Identifier: 10.3389/fninf.2016.00006
Volume: 10 Issue: First Page #:
Date Submitted: 9/7/17 12:00AM Date Published: 2/1/16 5:00AM
Publication Location:
Article Title: moco: Fast Motion Correction for Calcium Imaging
Authors: Alexander Dubbs, James Guevara, Rafael Yuste
Keywords: Machine Vision Algorithms 150.1135; calcium imaging; dynamic programming; fourier transform; mesoscale neuroscience; motion correction
Abstract: Motion correction is the first step in a pipeline of algorithms to analyze calcium imaging videos and extract biologically relevant information, for example the network structure of the neurons therein. Fast motion correction is especially critical for closed-loop activity triggered stimulation experiments, where accurate detection and targeting of specific cells in necessary. We introduce a novel motion-correction algorithm which uses a Fourier-transform approach, and a combination of judicious downsampling and the accelerated computation of many L 2 norms using dynamic programming and two-dimensional, fft-accelerated convolutions, to enhance its efficiency. Its accuracy is comparable to that of established community-used algorithms, and it is more stable to large translational motions. It is programmed in Java and is compatible with ImageJ.
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Journal: Proceedings of the National Academy of Sciences
Publication Identifier Type: DOI Publication Identifier: 10.1073/pnas.1504451113
Volume: 113 Issue: 15 First Page #: 3938
Date Submitted: 9/7/17 12:00AM Date Published: 4/1/16 4:00AM
Publication Location:
Article Title: Modulation of nitrogen vacancy charge state and fluorescence in nanodiamonds using electrochemical potential
Authors: Sinan Karaveli, Ophir Gaathon, Abraham Wolcott, Reyu Sakakibara, Or A. Shemesh, Darcy S. Peterka,
Keywords: fluorescence microscopy; nanodiamond; nitrogen vacancy center; voltage indicator; voltage sensing
Abstract: The negatively charged nitrogen vacancy (NV(-)) center in diamond has attracted strong interest for a wide range of sensing and quantum information processing applications. To this end, recent work has focused on controlling the NV charge state, whose stability strongly depends on its electrostatic environment. Here, we demonstrate that the charge state and fluorescence dynamics of single NV centers in nanodiamonds with different surface terminations can be controlled by an externally applied potential difference in an electrochemical cell. The voltage dependence of the NV charge state can be used to stabilize the NV(-) state for spin-based sensing protocols and provides a method of charge state-dependent fluorescence sensing of electrochemical potentials. We detect clear NV fluorescence modulation for voltage changes down to 100 mV, with a single NV and down to 20 mV with multiple NV centers in a wide-field imaging mode. These results suggest that NV centers in nanodiamonds could enab
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Publication Location:
Article Title: Imprinting and recalling cortical ensembles
Authors: L. Carrillo-Reid, W. Yang, Y. Bando, D. S. Peterka, R. Yuste
Keywords: optogenetics, cortical ensembles
Abstract: Neuronal ensembles are coactive groups of neurons that may represent building blocks of cortical circuits. These ensembles could be formed by Hebbian plasticity, whereby synapses between coactive neurons are strengthened. Here we report that repetitive activation with two-photon optogenetics of neuronal populations from ensembles in the visual cortex of awake mice builds neuronal ensembles that recur spontaneously after being imprinted and do not disrupt preexisting ones. Moreover, imprinted ensembles can be recalled by single-cell stimulation and remain coactive on consecutive days. Our results demonstrate the persistent reconfiguration of cortical circuits by two-photon optogenetics into neuronal ensembles that can perform pattern completion.
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Article Title: In vivo imaging of neural activity
Authors: Weijian Yang, Rafael Yuste
Keywords: in vivo functional imaging
Abstract: Since the introduction of calcium imaging to monitor neuronal activity with single-cell resolution, optical imaging methods have revolutionized neuroscience by enabling systematic recordings of neuronal circuits in living animals. The plethora of methods for functional neural imaging can be daunting to the nonexpert to navigate. Here we review advanced microscopy techniques for in vivo functional imaging and offer guidelines for which technologies are best suited for particular applications.
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Article Title: Imaging and Optically Manipulating Neuronal Ensembles
Authors: Luis Carrillo-Reid, Weijian Yang, Jae-eun Kang Miller, Darcy S. Peterka, Rafael Yuste
Keywords: calcium; optogenetics; two-photon
Abstract: The neural code that relates the firing of neurons to the generation of behavior and mental states must be implemented by spatiotemporal patterns of activity across neuronal populations. These patterns engage selective groups of neurons, called neuronal ensembles, which are emergent building blocks of neural circuits. We review optical and computational methods, based on two-photon calcium imaging and two-photon optogenetics, to detect, characterize, and manipulate neuronal ensembles in three dimensions. We review data using these methods in the mammalian cortex that demonstrate the existence of neuronal ensembles in the spontaneous and evoked cortical activity in vitro and in vivo. Moreover, two-photon optogenetics enable the possibility of artificially imprinting neuronal ensembles into awake, behaving animals and of later recalling those ensembles selectively by stimulating individual cells. These methods could enable deciphering the neural code and also be used to understand the pa
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Article Title: Electron Microscopic Reconstruction of Functionally Identified Cells in a Neural Integrator
Authors: Ashwin Vishwanathan, Kayvon Daie, Alexandro D. Ramirez, Jeff W. Lichtman, Emre R.F. Aksay, H. Seb
Keywords: zebrafish, two-photon microscopy, serial section electron microscopy, connectomic, eye-positionneural integrator, positive feedback
Abstract: Neural integrators are involved in a variety of sensorimotor and cognitive behaviors. The oculomotor system contains a simple example, a hindbrain neural circuit that takes velocity signals as inputs and temporally integrates them to control eye position. Here we investigated the structural underpinnings of temporal integration in the larval zebrafish by first identifying integrator neurons using two-photon calcium imaging and then reconstructing the same neurons through serial electron microscopic analysis. Integrator neurons were identified as those neurons with activities highly correlated with eye position during spontaneous eye movements. Three morphological classes of neurons were observed: ipsilaterally projecting neurons located medially, contralaterally projecting neurons located more laterally, and a population at the extreme lateral edge of the hindbrain for which we were not able to identify axons. Based on their somatic locations, we inferred that neurons with only ipsilat
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Article Title: Whole-brain serial-section electron microscopy in larval zebrafish
Authors: David Grant Colburn Hildebrand, Marcelo Cicconet, Russel Miguel Torres, Woohyuk Choi, Tran Minh Qu
Keywords: Classification and taxonomy, Cellular neuroscience, Imaging techniques, Characterization and analytical techniques, Data acquisition
Abstract: High-resolution serial-section electron microscopy (ssEM) makes it possible to investigate the dense meshwork of axons, dendrites, and synapses that form neuronal circuits. However, the imaging scale required to comprehensively reconstruct these structures is more than ten orders of magnitude smaller than the spatial extents occupied by networks of interconnected neurons, some of which span nearly the entire brain. Difficulties in generating and handling data for large volumes at nanoscale resolution have thus restricted vertebrate studies to fragments of circuits. These efforts were recently transformed by advances in computing, sample handling, and imaging techniques, but high-resolution examination of entire brains remains a challenge. Here, we present ssEM data for the complete brain of a larval zebrafish (Danio rerio) at 5.5 days post-fertilization. Our approach utilizes multiple rounds of targeted imaging at different scales to reduce acquisition time and data management requirem
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Article Title: Cell diversity and network dynamics in photosensitive human brain organoids
Authors: Giorgia Quadrato, Tuan Nguyen, Evan Z. Macosko, John L. Sherwood, Sung Min Yang, Daniel R. Berge
Keywords: Stem cells in the nervous system Induced pluripotent stem cells Neuroscience
Abstract: In vitro models of the developing brain such as three-dimensional brain organoids offer an unprecedented opportunity to study aspects of human brain development and disease. However, the cells generated within organoids and the extent to which they recapitulate the regional complexity, cellular diversity and circuit functionality of the brain remain undefined. Here we analyse gene expression in over 80,000 individual cells isolated from 31 human brain organoids. We find that organoids can generate a broad diversity of cells, which are related to endogenous classes, including cells from the cerebral cortex and the retina. Organoids could be developed over extended periods (more than 9 months), allowing for the establishment of relatively mature features, including the formation of dendritic spines and spontaneously active neuronal networks. Finally, neuronal activity within organoids could be controlled using light stimulation of photosensitive cells, which may offer a way to probe the
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Article Title: Efficiency of Cathodoluminescence Emission by Nitrogen-Vacancy Color Centers in Nanodiamonds
Authors: Huiliang Zhang, David R. Glenn, Richard Schalek, Jeff W. Lichtman, Ronald L. Walsworth
Keywords: cathodoluminescence; correlative microscopy; nanodiamond; nitrogen-vacancy; scanning electron microscopy
Abstract: Correlated electron microscopy and cathodoluminescence (CL) imaging using functionalized nanoparticles is a promising nanoscale probe of biological structure and function. Nanodiamonds (NDs) that contain CL-emitting color centers are particularly well suited for such applications. The intensity of CL emission from NDs is determined by a combination of factors, including particle size, density of color centers, efficiency of energy deposition by electrons passing through the particle, and conversion efficiency from deposited energy to CL emission. This paper reports experiments and numerical simulations that investigate the relative importance of each of these factors in determining CL emission intensity from NDs containing nitrogen-vacancy (NV) color centers. In particular, it is found that CL can be detected from NV-doped NDs with dimensions as small as ?40 nm, although CL emission decreases significantly for smaller NDs.
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Article Title: Similar synapse elimination motifs at successive relays in the same efferent pathway during development in mice
Authors: Shu-Hsien Sheu, Juan Carlos Tapia, Shlomo Tsuriel, Jeff W Lichtman
Keywords: mouse; neuroscience; parasympathetic nerves; peripheral ganglia; synapse elimination; synaptic competition
Abstract: In many parts of the nervous system, signals pass across multiple synaptic relays on their way to a destination, but little is known about how these relays form and the function they serve. To get some insight into this question we ask how the connectivity patterns are organized at two successive synaptic relays in a simple, cholinergic efferent pathway. We found that the organization at successive relays in the parasympathetic nervous system strongly resemble each other despite the different embryological origin and physiological properties of the pre- and postsynaptic cells. Additionally, we found a similar developmental synaptic pruning and elaboration strategy is used at both sites to generate their adult organizations. The striking parallels in adult innervation and developmental mechanisms at the relays argue that a general strategy is in operation. We discuss why from a functional standpoint this structural organization may amplify central signals while at the same time maintain
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Partners

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I certify that the information in the report is complete and accurate:
Signature: Rafael Yuste
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Columbia University
Frederick Gregory, Program Manager
Army Research Office
Text

Specifics and Impact

- Kickoff November 2012, Mid Review August 2014
- No cost extension till February 2015, funding till 8/2018
- Personnel: 11 postdocs, 8 graduate students, 2 technicians
- Groups meetings: monthly presentation with WebEx
- Publications: 45 peer-reviewed, several in top journals
- Nanodiamond MURI spinoff
- DARPA contracts to 6 PIs on MURI-seeded activities
- IARPA contract to 1 PI on MURI-seeded activities
- Honors: Lichtman, Sahin, Hillman, Min, Paninski, Yuste, Zhang
- MURI-inspired White House BRAIN Initiative now at $300M/yr
- Neurotechnology Center @ Columbia