

PI: Adesnik, Hillel	Title: All holographic two-photon electrophysiology	
Received: 05/04/2021	FOA: NS18-020	Council: 01/2022
Competition ID: FORMS-F	FOA Title: BRAIN Initiative: New Technologies and Novel Approaches for Large-Scale Recording and Modulation in the Nervous System (R01 Clinical Trials Not Allowed)	
1 R01 NS126070-01	Dual: AA,AG,AT,DA,DC,EB,EY,HD,MH	Accession Number: 4575451
IPF: 577502	Organization: UNIVERSITY OF CALIFORNIA BERKELEY	
Former Number:	Department: Molecular and Cell Biology	
IRG/SRG: ZNS1 SRB-O (16)	AIDS: N	Expedited: N
<u>Subtotal Direct Costs</u> <u>(excludes consortium F&A)</u> Year 1: 1,106,961 Year 2: 812,942 Year 3: 855,148	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	Organization:	Role Category:
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Stephen Brohawn	The Regents of the University of California	Co-Investigator
Laura Waller	The Regents of the University of California	Co-Investigator
Rikky Muller	The Regents of the University of California	Co-Investigator
Michael Lin	Stanford University School of Medicine	Other (Specify)-Subaward PI

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

		3. DATE RECEIVED BY STATE	State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier	
<input type="radio"/> Pre-application	<input checked="" type="radio"/> Application	<input type="radio"/> Changed/Corrected Application	b. Agency Routing Number
2. DATE SUBMITTED	Application Identifier	c. Previous Grants.gov Tracking Number	
5. APPLICANT INFORMATION			Organizational DUNS* : 1247267250000
Legal Name*:	The Regents of the University of California		
Department:	Sponsored Projects Office		
Division:			
Street1*:	1608 Fourth Street, Suite 220		
Street2:	University of California, Berkeley		
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County:	Alameda		
State*:	CA: California		
Province:			
Country*:	USA: UNITED STATES		
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6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)* 1946002123A1			
7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education			
Other (Specify):			
<input checked="" type="checkbox"/> Small Business Organization Type <input type="radio"/> Women Owned <input type="radio"/> Socially and Economically Disadvantaged			
8. TYPE OF APPLICATION*		If Revision, mark appropriate box(es).	
<input checked="" type="radio"/> New	<input type="radio"/> Resubmission	<input type="radio"/> A. Increase Award	<input type="radio"/> B. Decrease Award
<input type="radio"/> Renewal	<input type="radio"/> Continuation	<input type="radio"/> C. Increase Duration	<input type="radio"/> D. Decrease Duration
	<input type="radio"/> Revision	<input type="radio"/> E. Other (specify):	
Is this application being submitted to other agencies?*		<input type="radio"/> Yes	<input checked="" type="radio"/> No
All holographic two-photon electrophysiology		What other Agencies?	
9. NAME OF FEDERAL AGENCY* National Institutes of Health		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER TITLE:	
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT* All holographic two-photon electrophysiology			
12. PROPOSED PROJECT Start Date* 02/01/2022		13. CONGRESSIONAL DISTRICTS OF APPLICANT Ending Date* 01/31/2025	
CA-013			

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

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15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	\$3,997,946.00
b. Total Non-Federal Funds*	\$0.00
c. Total Federal & Non-Federal Funds*	\$3,997,946.00
d. Estimated Program Income*	\$0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

- a. YES THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
 DATE:
- b. NO PROGRAM IS NOT COVERED BY E.O. 12372; OR
 PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

I agree*

* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION

File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Margaret Middle Name: Last Name*: Nguyen Suffix:
 Position>Title: Contract and Grant Officer
 Organization Name*: The Regents of the University of California
 Department: Sponsored Projects Office
 Division:
 Street1*: 1608 Fourth Street, Suite 220
 Street2: University of California, Berkeley
 City*: Berkeley
 County: Alameda
 State*: CA: California
 Province:
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Signature of Authorized Representative*

Margaret Nguyen

Date Signed*

05/04/2021

20. PRE-APPLICATION File Name:**21. COVER LETTER ATTACHMENT** File Name:

424 R&R and PHS-398 Specific

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Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The Regents of the University of California
Duns Number: 1247267250000
Street1*: Weill Hall, #205
Street2: University of California, Berkeley
City*: Berkeley
County: Alameda
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 94720-3200
Project/Performance Site Congressional District*: CA-013

Project/Performance Site Location 1

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Stanford University
DUNS Number: 0092142140000
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City*: Stanford
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 943050000
Project/Performance Site Congressional District*: CA-018

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* Yes No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes NoIf YES, check appropriate exemption number: 1 2 3 4 5 6 7 8If NO, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number D1600050

3. Is proprietary/privileged information included in the application?* Yes No**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an Yes No environmental assessment (EA) or environmental impact statement (EIS) been performed?

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* Yes No

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* 29639_Abstract_FINAL.pdf**8. Project Narrative*** 29639_Narrative_FINAL.pdf**9. Bibliography & References Cited** 29639_Bibliography_FINAL.pdf**10. Facilities & Other Resources** 29639_Facilities_FINAL.pdf**11. Equipment** 29639_Equipment_FINAL.pdf

PROJECT SUMMARY

Achieving a detailed understanding of the neural codes of sensation, action and cognition will require technologies that can both sample and perturb neural activity with millisecond precision and cellular resolution across large populations of neurons. We will develop an all-optical all-holographic read/write two photon microscope that can both simultaneously record and perturb population neural activity with cellular resolution and millisecond precision. To achieve this, we will develop multispectral temporally focused three-dimensional (3D) wavefront shaping. This new form of hybrid two photon microscopy will enable simultaneous illumination of user-defined ensembles of neurons in 3D with cell-size spots of two photon excitation light at two different wavelengths – one for imaging a voltage sensor and one for controlling neural activity optogenetically. The two holographic optical paths will be completely independent and update at >kilohertz speed with the ability sample and perturb the membrane voltage of tens to hundreds of neurons at a time. In tandem, we will develop task-optimized two-photon excitable red-shifted genetically encoded voltage sensors (GEVIs) and ultrapotent blue-shifted microbial opsins that are spectrally separable. The development of an all-holographic read/write microscope will permit neuronal recording and perturbation on spatial and temporal scales that are currently well beyond reach which could substantially facilitate systems neuroscience research.

PROJECT NARRATIVE

Understanding how spatiotemporal patterns of neural activity mediate brain functions requires new approaches to monitor and manipulate neural activity with much greater spatial and temporal resolution, at greater scale, and deeper into the brain than what is currently possible. To overcome the limits of existing technologies, this BRAIN initiative application proposes to develop a new neuro-imaging platform that can both measure and perturb neuronal activity with millisecond precision and cellular resolution which could address brain function in health and disease with unprecedented detail.

FACILITIES AND OTHER RESOURCES – University of California, Berkeley

The Adesnik Lab comprises 2330 sq. ft. of dedicated lab space in the Weill Hall on UC Berkeley's main campus. It has two dedicated multiphoton holographic light microscopes for *in vivo* imaging of the rodent brain under awake, head-fixed conditions and holographic optogenetics. Both of the multiphoton microscopes are additionally equipped with an imaging laser, Coherent Ultra II Ti:Sapphire, and a fixed wavelength fiber laser (Satsuma HP or Coherent Monaco). A conventional white light source is used for wide field excitation. Both rigs are also equipped for single cell electrophysiology (Axon Multiclamp, Sutter MP-285 micromanipulator). All microscopes run off a combination of custom and open source software (ScanImage – Janelia Farms).

There are two separate stereotaxic injection rigs for viral transfection and animal surgery, and a full suite of equipment necessary to carry out histological analysis of tissue from transgenic mouse lines. The laboratory is equipped with 24 PC computers, a backup server and two nVidia TESLA GPUs for data processing. There is a complete intrinsic optical imaging rig, a fluorescent macroscope, and all the equipment for standard molecular cloning and PCR based genotyping. We have additional rigs equipped for intracellular patch clamp *in vivo* and in brain slices, and multi-electrode array recordings *in vivo*. All rigs are controlled by either commercially available or custom in-house software for data acquisition, sensory stimulation, and data analysis.

Animals: Mice are housed in the AAALAC-approved vivaria maintained by the Office of Laboratory Animal Care (OLAC) with oversight by the UC Berkeley Animal Care and Use Committee in Weill Hall. The Adesnik lab has sufficient space to maintain the transgenic breeding colonies needed for the proposed work. All breeding and surgeries are planned to take place in the animal research space currently assigned to Dr. Adesnik in the Life Science Addition Building.

Other space: UC Berkeley's Molecular Imaging Center maintains a number of high-performance microscopes accessible for this proposal.

Computation Resources: The Adesnik lab (as well as that of Dr. Waller and Dr. Ji) has access to UC Berkeley's high performance computer cluster for fast parallelization of data analysis. The computer cluster is used extensively both for online generation of computer-generated holograms and for post-hoc analysis of all neural data.

The Ji Lab is located in the newly established Li Ka Shing (LKS) Center for biomedical and health science on UC Berkeley's main campus. This is a new 200,000 sq. ft. research facility that includes over 30 research and teaching laboratories as well as state of the art auditoriums, lecture halls, conference rooms and educational facilities. The Ji lab has ~2000 sq. ft. of dedicated lab space in the Li Ka Shing (LKS) building, and \$1.9M equipment including five ultrafast laser systems (two Chameleon Ultra II from Coherent, one Monaco-OperaF system from Coherent, one Insight DeepSee from Spectra Physics, one optical parametric oscillator from Coherent), two home-built adaptive optical two-photon fluorescence microscopes, one home-built adaptive optical three-photon fluorescence microscope, and an Olympus microscope. The Ji lab has extensive experiences with phase modulation devices, and is equipped with four liquid-crystal spatial light modulators (two from Holoeye, one from Boulder Nonlinear Systems, and one from Meadowlark) and four deformable mirrors (two from Iris AO, two from Boston Micromachines). The Ji lab has an intracellular patch clamp setup (Axon Instruments) for investigations *in vivo* and in brain slices. All equipment is controlled by either commercially available or custom in-house software for data acquisition, sensory stimulation, and data analysis. The lab has two stereotaxic injection rigs and dissection scopes for viral transfection and animal surgery, and a full suite of equipment necessary to carry out histological analysis of tissue.

The Brohawn Lab is fully equipped for experiments related to rational engineering of variant opsins including ion channel expression, purification, reconstitution, functional recording, and structure determination. The laboratory consists of ~1800 sq. ft. of space including ten benches, a tissue culture room, an electrophysiology room, a crystallography/EM room, an equipment room, a computer room, and cold room. The lab also has access to extensive instrumentation and facilities resources at UC Berkeley. This includes additional crystallization robots, a room temperature crystal storage and imaging hotel, a Vitrobot for preparation of frozen samples for EM, and microscopes suitable for negative staining and single particle cryo-EM including a Titan Krios with direct electron detector and energy filter. The lab has access to the X-ray beamline 8.3.1 at the nearby ALS synchrotron and to microfocus beamlines at GM-CAT at the APS synchrotron. The Brohawn lab is located in Weill Hall with primary affiliations in The Helen Wills Neuroscience Institute and Department of Molecular and Cell Biology Neurobiology Division and a secondary affiliation with the division of Biochemistry,

Biophysics, and Structural Biology. The lab interacts with leading labs in neuroscience and ion channel function and mechanism (including Ehud Isacoff, Diana Bautista, Marla Feller, Dan Feldman, Richard Kramer, Helen Bateup, Stephan Lammel, and Polina Lishko) and the structure and function of biomolecules (including Andy Martin, Jim Hurley, John Kuriyan, Karen Davies, and Eva Nogales).

The Waller Lab has an extensive optics lab space in Cory Hall with six vibration isolated optical tables and a wide variety of microscopes (Nikon TE300 x2, Nikon AZ100, several custom-built microscopes), filters, lasers, cameras, spatial light modulators, lenses, beamsplitters, opto-mechanics and other basic lab parts. The Waller group has a membership to the UC Berkeley Innovation Lab, which has excellent shared facilities for 3D printing, laser cutting and mechanical machining of parts. We also have access to the UC Berkeley Marvel Nanolabs for microfabrication purposes, where necessary. In the lab are several calibrated MEMS printed surfaces and biological samples for testing of our imaging systems with well-characterized samples.

The Muller Lab has dedicated laboratory space in Cory Hall, including a 500-sqft laboratory for electronic testing. The Muller lab further has access to a shared 960-sqft wet chemical and biologic laboratory, which includes fume hoods, biological hoods and sinks.

Marvell Nanofabrication Facility: UC Berkeley Marvell Nanolab is the only facility on the UC Berkeley campus that provides research space and knowledge in state-of-the-art semiconductor and nanofabrication technology. Among the laboratory's major features are a computer area for device and circuit layout; a lithography center, which includes three step-and-repeat reduction cameras, two contact aligners, and a mask-making facility with an optical pattern generator; thin-film systems; 6" silicon VLSI wafer processing areas equipped with LPCVD and atmospheric furnaces, process-specific plasma etchers, wet processing stations, and in-line testing and analytical diagnostics; a planarization laboratory with chemical-mechanical polish and interlayer dielectric deposition; a satellite thin-film lab housing a 5-chamber high-vacuum cluster tool for dc-magnetron sputtering of metal-based thin-films. The facility supports a 0.35 μm CMOS baseline process. The Device Characterization Laboratory is equipped with manual probe stations and an automated wafer probe station. The list of major and capital equipment for micro and nanofabrication at the Marvell Laboratory can be found here: <https://nanolab.berkeley.edu/public/equipment/EquipmentAllActive.pdf>

Qualcomm Swarm lab (4th floor Cory Hall): The Swarm Lab contains several thousand square feet of combined electronics testing, plastic rapid prototyping machines, microscopy, general purpose lab space, computing infrastructure, office and conference space.

FACILITIES AND OTHER RESOURCES – Stanford University

The Lin Lab is located the first floor of new Neuroscience Building, completed in 2019. The Lin lab space includes benches for 18 scientists with adjacent desks, a tissue culture room, and a microscopy room, assigned to the Principal Investigator as independent space. The laboratory has machines for performing molecular biology procedures including thermal cyclers, centrifuges, electrophoresis systems, incubators, and nano-volume and microplate spectrophotometers. The laboratory has 4 epifluorescence microscopes for fluorescence imaging of cells and sections, each equipped with a broad-spectrum fluorescence excitation light source, six filter sets covering the visible spectrum, motorized XY stages and Z focus, and a high-sensitivity CCD camera, all placed under programmable control. The laboratory also has a Perkin-Elmer Yokogawa spinning-disk confocal system on a Zeiss Axio Observer with 4 laser excitation lines. All procedures relating to gene expression in mammalian cells in the Lin Lab are authorized to be performed in the tissue culture room under an existing BSL2+ biosafety protocol. The lab also has equipment for culturing neurons and non-neuronal cells in the form of a biosafety lamina flow hood, CO₂ incubators, centrifuges, dissecting microscopes, and electroporators.

Office: Dr. Lin has his own office of about 200 sq ft adjacent to the lab. All members of the Lin Lab have their own desks adjacent to the lab. There is a color printer, photocopier, fax machine, scanner, and general-use computer in the shared administrative area outside Dr. Lin's office.

Computer: The laboratory has modern computer systems complete with operating system, word processing, communication, image analysis, and presentation software, networked together for efficient file exchange. Free technical support is provided by a university computer help office via email or telephone. Free on-site technical

support including hardware and software installation and repairs is provided by a full-time technician in the Neuroscience Building. When necessary, updates to operating system, word processing, communication, presentation software, and scientific software, will be purchased at discounted site license prices from the university purchasing department. ImageJ is used for all image analysis and quantification, and is available for free from the NIH website.

Scientific Environment: The Neuroscience building is adjacent to the Stanford School of Medicine, which contains the research facilities of all 19 medical departments as well as 9 basic science departments. The proximity of the research buildings, the juxtaposition of laboratories of different departments in each building, and the centralization of conference facilities fosters research synergies and exceptionally efficient communication. The Lin Lab also actively participates in seminars organized by the departments of Chemical Biology, Developmental Biology, Neurobiology, Pediatrics, and Bioengineering featuring internal speakers to further facilitate intellectual exchange and collaboration. As discussed further below, comprehensive support in the form of animal and core facilities is available from facilities located in the Neuroscience Building and adjacent buildings. The environment is therefore very well optimized for the success of the project.

Other: Available technical core services that are available at Stanford and are relevant to the proposed work include transgenic mouse production, live cell microscopy, and adeno-associated virus production. These services are performed by full time staff and offered on a fee-for-service basis.

RESEARCH ENVIRONMENT

UC Berkeley offers a highly interactive, intellectually rich, and diverse scientific community. There are numerous seminar series and research meetings in many areas related to our research, including talks on neurobiology, optical imaging, and data analytics.

The Adesnik and Brohawn labs are located on the second floor of Weill Hall, which is occupied by members of Molecular and Cell Biology department. There are several additional neuroscience and imaging labs in the immediate vicinity that share the Adesnik's lab interest in understanding sensory function in the mammalian nervous system and will benefit from the new technology, including Marla Feller, Richard Kramer, and Diana Bautista. The Ji lab is located in the Li Ka Shing (LKS) building about a 5 minute walk from the Life Sciences Addition building. Drs. Adesnik, Ji, and Brohawn, are members of the Helen Wills Neuroscience Institute at Berkeley which brings together faculty from a number of different departments who focus on basic questions in neuroscience research. The Waller and Muller labs are located in Cory Hall next to several optics labs in the Electrical Engineering and Computer Science department (e.g. Profs. Ming Wu, Connie Chang-Hasnain, Eli Yablonovitch). The collaborative environment within the EECS department makes it easy for labs to share equipment, borrow tools, and help each other. The Waller Lab has significant software infrastructure and supports the data sharing and archiving of large datasets and code.

Stanford: The Lin laboratory presently comprises 6 post-doctoral fellows, 7 doctoral students, one technician and myself. In addition, as I have selected post-doctoral fellows from a range of intellectual backgrounds, ranging from cell biology and systems neuroscience into molecular and classical genetics in flies, the group has a rich, broad body of expertise which can both guide our research in novel directions, and solve problems that arise in any project from a number of creative perspectives. In addition, my laboratory is physically located in the Department of Neurobiology, a Department with a long tradition of strength in systems neuroscience, and our work benefits significantly with direct interactions with our colleagues Dr. Newsome, an expert in primate motion processing, and Dr. Baccus, an expert in retinal circuitry. More broadly, our group works within the Stanford Neuroscience community, where we interact frequently with Drs. Schnitzer, Deisseroth, and Goodman, all experts in neural circuit studies and behavior. Finally, within the Stanford community, as there are many faculty members with substantial interests in nervous system function, we have many seminar speakers and targeted symposia that address various aspects of neural circuit function, creating a rich intellectual environment in which this project can succeed.

EQUIPMENT – University of California, Berkeley

The Adesnik Lab possesses all instrumentation necessary for animal surgery and preparation proposed in this application. The Adesnik lab has the microscopes and electrophysiological equipment needed for proof-of-concept testing of 3D temporally focused multiphoton holography for imaging and optogenetics, including two complete dual laser multiphoton system (built on the Sutter MOM platform) with a Coherent Ultra II and a Coherent Monaco 40W laser or an Amplitude Systems 20W Satsuma laser, Meadowlark SLMs for 3D-SHOT as well as SLMs for fast remote focusing. Both systems are equipped with amplifiers and micromanipulators for simultaneous electrophysiological recording. A third non-scanning microscope equipped with a Spectra Physics Femtotrain (6W) laser will be used for validating GEVLs and opsins with two photon optogenetics. One of the Sutter MOM 3D-SHOT microscopes will be used for all the experiments in Aim 1 by equipping it with an EMCCD camera (Hnu Nuvu 128) for imaging. The second Sutter MOM 3D-SHOT microscope will be upgraded with an optical parametric amplifier pumped by an Amplitude Systems Tango 100W femtosecond laser system to provide necessary power (~10W) at a shorter wavelength for optogenetic stimulation and 1030 nm power for imaging. The system will be further equipped with a multianode PMT array (Hamamatsu) and the necessary readout electronics for detection (Vertilon). We will integrate custom designed MEMS-SLMs from the Muller lab (1-4 SLMs with temporal delays). This microscope will be used for the validation experiments performed in Aim 3. Both rigs are fully equipped for patch clamp electrophysiology where needed, and all the computer software and hardware to run the systems. The Adesnik lab also has a multielectrode array physiology rig, a blind patch clamp *in vivo* rig, and a one-photon structured illumination microscope. It also has all the necessary equipment for cell culture and basic molecular subcloning. Viruses will be prepared at the UC Berkeley AAV vector core.

The Ji Lab is also equipped with multiple multiphoton systems, one of which includes the necessary optical hardware for FACED kHz voltage imaging. The Waller lab has the optical equipment necessary to prototype the holographic approach and PMT array detection, simulate and test holographic computation methods on high performance computer workstations, and then test and validate it on the microscopes in the Adesnik lab. The Brohawn lab has all the necessary equipment for opsin mutagenesis, sub-cloning, cell culture expression, and electrophysiology. Multiphoton assessment of the new opsins will be tested on the Adesnik lab's brain slice microscope. The Brohawn lab has all the needed computing hardware for structural modeling and simulation of mutant opsins. The Muller lab has access to fabrication facilities at UC Berkeley to generate the MEMS-SLMS, as well as the software to design the systems. They have laser systems and cameras necessary to test their functionality prior to integration into microscopes. The Lin lab has all the molecular engineering equipment for high throughput modification and testing of new ASAP voltage sensor variants, as well the ability to test the variants in neuronal cell culture with wide field imaging prior to testing in the Adesnik and Ji labs *in vivo* at UC Berkeley.

The Brohawn Lab is fully equipped for molecular biology, biochemistry, structural biology, and electrophysiology. For protein expression, we have five Infors shaker incubators (two with CO₂) and a bioreactor for large-scale growth of mammalian, insect, yeast, and bacterial cells. For protein purification, we have a Retsch MM400 miller, a Branson cell disrupter, two Biorad NGC liquid chromatography systems, an Agilent HPLC with in-line fluorescence detector, a Sorvall Lynx superspeed centrifuge and rotors, a Beckman Optima ultracentrifuge and rotors, a Sorvall Legend benchtop centrifuge and rotors, and a Denovix DS11+ spectrophotometer. For cryo-EM sample preparation, we have a Vitrobot Mark IV plunge freezer and glow discharger in the lab and access to a shared Vitrobot Mark IV plunge freezer, a Leica GP2 plunge freezer, and glow dischargers. For cryo-EM data collection, we have dedicated access to two Titan Krios 300 kV microscopes with Gatan energy filters and a Talos Arctica 200 kV microscope, all with K3 direct electron detectors, on campus through the Cal-Cryo Instrumentation Core Facility and Bay Area Cryo-EM Facility. For crystallography, we have a Formulatrix NT8 drop-setting robot, a Formulator screen dispensing robot, and a RockImager 182 crystal screen imaging hotel in the lab and access to a shared TTP Mosquito drop-setting robot, a Rigaku imaging hotel, and beamtime at ALS and APS synchrotron beamlines. For computation, we have eleven custom-built high-end Linux workstations, each with multiple GPUs (4 Nvidia 1080Ti or 2-4 Nvidia 2080Ti), 16-32 core AMD Ryzen CPUs, 128-256 GB RAM, 1-4 TB fast SSD storage, and 50-200 TB RAID storage. We have three electrophysiology rigs for recording from reconstituted systems, cells, and brain slices with Axon 200B amplifiers and 1550B digitizers, Sutter micromanipulators, ALA pressure clamps, Automate perfusion systems, and TMC vibration isolation tables and Faraday cages. One rig has a Zeiss D1 microscope with Lumencor LED light engine, one rig has a Zeiss A1 microscope, and one rig has a Sutter microscope with Lumencor SpectraX light engine and infrared camera. We have a Sutter P1000 pipette puller and Narishige microforge. We have a standalone Nanion Orbit mini unit for

planar bilayer recordings. We have two Zeiss DV20 stereo microscopes for surgeries and dissections. We have a Molecular Devices Spectra Max M3 fluorescence plate reader for ion flux assays. Finally, we related small equipment including incubators for cell growth, desktop centrifuges, and DNA and protein electrophoresis setups.

The Muller Lab has dedicated laboratory space in Cory Hall, including a 500-sqft laboratory for electronic testing that includes low noise, high precision test equipment such as oscilloscopes, low noise power supplies, precision source-meters, a current waveform analyzer, LRC meter, high dynamic range signal sources, laboratory computers, server machines and more. The Muller lab further has access to a shared 960-sqft wet chemical and biologic laboratory, which includes fume hoods, biological hoods and sinks.

The Waller Lab has dedicated space in Cory Hall There are two high-precision (~5nm) motion stages and a rotation stage for computer-controlled motion of optical elements, three sCMOS cameras (~6 Mpxls, 100 fps, 16 bit) and a Texas Instruments DLP array with developer kit, as well as multiple Liquid Crystal (LCOS) spatial light modulators (SLMs). Opto-electronic equipment are connected to one of five custom-built high-performance desktop computers. Each computer has at least 128 Gb RAM and houses a state-of-the-art GPU processor (NVIDIA Tesla or K20 or better) which are programmed via free software (NVIDIA CUDA and C/Visual Studio). Sufficient ports for all of the experimental equipment that supports computer interfacing are available.

EQUIPMENT – Stanford University

The Lin Lab possesses a custom-built electrical screening system comprising an Olympus IX81 motorized inverted microscope with a 20x/0.75NA air objective, a motorized Ludl stage, high-power 470-nm LED illuminator, green and red filter cubes, a Grass generator, a programmable motor for electrode positioning, and a Hamamatsu Flash4.0 camera, all run by a custom Matlab program. The Lin lab also possesses an electrophysiology system comprising a Zeiss Axiovert 100M motorized inverted microscope with 20x/0.75NA air and 40x/1.1NA water objectives, high-power 470-nm LED illuminator, complete filter cubes, Andor iXon 860 EM-CCD, Axon Instruments AxoPatch amplifier and headstage, and Siskiyou micromanipulators, on a XY platform placed on a TMC anti-vibration table, controlled by a computer running pCLAMP and Micro-Manager software.

Other equipment that may be used for routine purposes include the following:

- Confocal microscope comprising a Zeiss Axio Observer motorized inverted microscope with 20x/0.75NA air, 40x/1.1NA water, and 60x/1.3NA oil objectives, Perkin-Elmer Ultraview confocal unit with Yokogawa CSU-X1 spinning disk unit and Hamamatsu Flash4.0 sCMOS camera, controlled by OpenLab acquisition software.
- Automated fluorescence microscopy system comprising FEI iMic motorized inverted microscope with 20x/.75NA and 40x/1.1NA water objectives, Sutter Lambda LS broad spectrum illuminator, filters in motorized turret, Hamamatsu Flash4.0 sCMOS camera, FEI XY stage, and FEI environmental chamber, controlled by a computer running FEI software.
- Two automated fluorescence microscopy systems comprising Olympus IX81 or Zeiss Axiovert 200M motorized inverted microscope with 20x/0.75NA and 40x/1.1NA water objectives, Exfo X-cite 120 broad spectrum illuminator, filters in motorized turret and filter wheel, Hamamatsu Orca-ER CCD camera, Ludl motorized XY stage, and LCI environmental chamber, controlled by a computer running Micro-Manager software.
- One EVOS FL fluorescence inverted microscope for routine tissue-culture imaging and rapid assessment of transfection efficiency or construct brightness.
- One standard and one fluorescence (Olympus SZX-12) dissecting microscope for preparation of neuronal cultures and visualization of slice or brain fluorescence.
- Two 8-ft laminar flow hoods for tissue culture work up to BSL2+.
- Tabletop centrifuge with swinging-bucket rotor for tissue-culture and microcentrifuges for molecular biology.
- Amaxa Nucleofector and Lifetech Neon electroporation machines for neuronal transfections.
- TECAN Infinite M1000 PRO multiwell absorbance and fluorescence plate reader spectrometer with nanoliter read capability.

- Thermo Nanodrop 1000 absorbance spectrophotometer with nanoliter read capability, located in CCSR 2100.
- Four Techne TC-series thermal cyclers and one Life Technologies ProFlex thermal cycler. DNA gel apparatuses and imaging cabinet.
- Tissue-culture incubators.
- Bacterial culture incubators and shaker.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Hillel	Middle Name	Last Name*: Adesnik	Suffix:
Position/Title*:	Associate Professor			
Organization Name*:	The Regents of the University of California			
Department:	Molecular and Cell Biology			
Division:				
Street1*:	Weill Hall, #201			
Street2:	University of California, Berkeley			
City*:	Berkeley			
County:	Alameda			
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	94720-3200			
Phone Number*:	510/642-2107		Fax Number:	
E-Mail*:	hadesnik@berkeley.edu			
Credential, e.g., agency login:	HADESNIK			
Project Role*:	PD/PI		Other Project Role Category:	
Degree Type:	PHD,BA		Degree Year: 2007,2002	
Attach Biographical Sketch*:	File Name:	29639_Biosketch_Adesnik.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person

Prefix:	First Name*: N	Middle Name	Last Name*: Ji	Suffix:
Position/Title*:	Associate Professor			
Organization Name*:	The Regents of the University of California			
Department:	Molecular and Cell Biology			
Division:				
Street1*:	Li Ka Shing Center, #280			
Street2:	University of California, Berkeley			
City*:	Berkeley			
County:	Alameda			
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	94720-3370			
Phone Number*: 571-439-0934		Fax Number:		
E-Mail*: jina@berkeley.edu				
Credential, e.g., agency login: simplive				
Project Role*: Co-Investigator		Other Project Role Category:		
Degree Type: PHD		Degree Year: 2005		
Attach Biographical Sketch*:		File Name:	29639_Biosketch_Ji.pdf	
Attach Current & Pending Support: File Name:				

PROFILE - Senior/Key Person

Prefix:	First Name*: Stephen	Middle Name Graf	Last Name*: Brohawn	Suffix:
Position/Title*:	Assistant Professor of Neurobiology			
Organization Name*:	The Regents of the University of California			
Department:	Molecular and Cell Biology			
Division:				
Street1*:	Weill Hall, #289			
Street2:	University of California, Berkeley			
City*:	Berkeley			
County:	Alameda			
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	94720-3200			
Phone Number*: 617-800-7409		Fax Number:		
E-Mail*: brohawn@berkeley.edu				
Credential, e.g., agency login: sbrohawn				
Project Role*: Co-Investigator		Other Project Role Category:		
Degree Type: PHD,BS		Degree Year: 2010,2004		
Attach Biographical Sketch*:		File Name:	29639_Biosketch_Brohawn.pdf	
Attach Current & Pending Support: File Name:				

PROFILE - Senior/Key Person

Prefix:	First Name*: Laura	Middle Name	Last Name*: Waller	Suffix:
Position/Title*:	Associate Professor			
Organization Name*:	The Regents of the University of California			
Department:	Electrical Engineering & CSci			
Division:				
Street1*:	Cory Hall, #253			
Street2:	University of California, Berkeley			
City*:	Berkeley			
County:	Alameda			
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	94705-0000			
Phone Number*: 617-417-7909		Fax Number:		
E-Mail*: waller@berkeley.edu				
Credential, e.g., agency login: Waller				
Project Role*: Co-Investigator		Other Project Role Category:		
Degree Type: PHD,M ENG,BS		Degree Year: 2010,2005,2004		
Attach Biographical Sketch*:		File Name:	29639_Biosketch_Waller.pdf	
Attach Current & Pending Support: File Name:				

PROFILE - Senior/Key Person

Prefix:	First Name*: Rikky	Middle Name	Last Name*: Muller	Suffix:
Position/Title*:	Assistant Professor			
Organization Name*:	The Regents of the University of California			
Department:	Electrical Engineering & CSci			
Division:				
Street1*:	Soda Hall, #387			
Street2:	University of California, Berkeley			
City*:	Berkeley			
County:	Alameda			
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	94720-1776			
Phone Number*: 510-642-1042		Fax Number:		
E-Mail*: rikky@berkeley.edu				
Credential, e.g., agency login: rikkymuller1				
Project Role*: Co-Investigator		Other Project Role Category:		
Degree Type: PHD,MS,BS		Degree Year: 2013,2004,2004		
Attach Biographical Sketch*:		File Name:	29639_Biosketch_Muller.pdf	
Attach Current & Pending Support: File Name:				

PROFILE - Senior/Key Person

Prefix:	First Name*: Michael	Middle Name Z.	Last Name*: Lin	Suffix:
Position/Title*:	Associate Professor			
Organization Name*:	Stanford University School of Medicine			
Department:	Neurobiology & Bioengineering			
Division:				
Street1*:	290 Jane Stanford Way			
Street2:	ChemH-Neuro Complex 287, Mailcode 5090			
City*:	Stanford			
County:				
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	943050000			
Phone Number*:	650-721-1681		Fax Number:	
E-Mail*:	mzlin@stanford.edu			
Credential, e.g., agency login: LIN.MICHAEL				
Project Role*:	Other (Specify)	Other Project Role Category: Subaward PI		
Degree Type:	MD,PHD,BA	Degree Year: 2004,2002,1994		
Attach Biographical Sketch*:	File Name:	29639_Biosketch_Lin.pdf		
Attach Current & Pending Support:	File Name:			

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Adesnik, Hillel Azriel

eRA COMMONS USER NAME (credential, e.g., agency login): hadesnik

POSITION TITLE: Associate Professor of Neurobiology

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Columbia University, New York	B.A.	06/2002	Biology
University of California, San Francisco	Ph.D.	06/2007	Neuroscience
University of California, San Diego	Postdoctoral	12/2011	Neuroscience

A. Personal Statement

My research addresses the synaptic, circuit and network basis of sensory perception. I have a broad background in neurophysiology and optical methods, with specific training and expertise in investigating neuronal circuits as the basis of neural computation. My laboratory has extensive experience with multiphoton imaging and both one and two photon optogenetics in rodents. We have spent the last ten years developing new optical advances in multiphoton holography, modifying and engineering microbial opsins for multiphoton optogenetics, and developing computational tools for calibrating, controlling, and analyzing data from multiphoton holographic microscopes. As a graduate student and as a postdoctoral fellow I developed novel optical and electrophysiological approaches to probe the function of synapses and circuits in the brain. I have over 18 years of experience working on the physiology of circuits of the mammalian central nervous system which has led to many peer-reviewed publications based on original research, many in high impact journals. I have an extensive track record pioneering new optical and electrophysiological approaches to address key questions in neuroscience. I also have extensive experience collaborating with members of other laboratories with complementary expertise, such as in computation, optics, and electrical engineering, to make critical advances in neurotechnology. I have trained over 15 postdoctoral fellows – three currently in independent faculty positions, and 7 graduate students.

B. Positions and Honors

Positions and Employment

- 2002-2007 Ph.D. student, University of California, San Francisco, Laboratory of Roger Nicoll
2008-2011 Postdoctoral Fellow, University of California, San Diego, Laboratory of Massimo Scanziani
2012-2018 Assistant Professor of Neurobiology, University of California, Berkeley
2018-present Associate Professor of Neurobiology, University of California, Berkeley

Other Experience and Professional Memberships

- 2002-2007 Howard Hughes Medical Institute Predoctoral Fellowship
2002 National Science Foundation Predoctoral Fellowship
2008-2011 Helen Hay Whitney Postdoctoral Fellowship
2011- Society for Neuroscience

Awards

2016	President's early career award for scientists and engineers (PECASE)
2016	American Association of Anatomists Young Investigator Award
2015	New York Stem Cell Foundation Robertson Investigator
2013	NIH New Innovator Award
2013	McKnight Scholars Award
2013	Beckman Young Investigator Award
2013	Pew Biomedical Scholarship (awarded but declined)
2012	Whitehall Foundation Fellowship
2012	Winkler Family Foundation Fellowship
2008	Helen Hay Whitney Postdoctoral Fellowship
2002	Howard Hughes Medical Institute Predoctoral Fellowship
2002	National Science Foundation Graduate Research Fellowship (awarded but declined)
2001	Goldwater Foundation Scholarship

C. Contribution to Science

1. **Synaptic and circuit basis of visual computation:** This line of research addresses the neural mechanisms of sensory computation in the visual cortex. Using a combination of intracellular and extracellular recording, optogenetics and imaging we have discovered novel circuit motifs in rodent visual cortex that contribute to critical aspects of neural coding, with a focus on contextual modulation. In particular, we have focused on the synaptic and circuit mechanisms of surround suppression and contrast gain control, as well as on the role of inhibitory circuits in neuronal synchronization for contextual stimuli.
 - a) Synaptic mechanisms of feature coding in the visual cortex of awake mice. Adesnik H. *Neuron* 2017 Aug 30;95(5):1147-1159.e4. PMID: PMC5580349
 - b) Cortical gamma band synchronization through somatostatin interneurons. Veit J, Hakim R, Jadi MP, Sejnowski TJ, Adesnik H. *Nature Neuroscience* 2017 May 8. PMCID: PMC5511041
 - c) Adesnik, H., Bruns, W., Taniguchi, H., Huang, Z.J., Scanziani M. A Neural Circuit for Spatial Summation in the Visual Cortex. *Nature* 2012 490(7419):226-31. PMCID: PMC3621107
 - d) Olsen, S., Bortone, D., Adesnik, H., Scanziani, M. (2012) Gain Control by Cortical Layer 6 in the Visual Cortex. *Nature* 482:47-52 PMID: 22367547
2. **Probing the roles of specific cortical layers in sensory coding:** These studies investigate how each of the cortical layers and layer-specific inhibitory circuits control sensory coding in the cortex.
 - a) A direct translaminar inhibitory circuit tunes cortical output. Pluta, S., Naka, A., Veit, J., Telian, G., Hakim, R., Adesnik, H. *Nature Neuroscience* 2015 Nov;18(11):1631-40 PMCID: PMC4624464
 - b) Complementary networks of cortical somatostatin interneurons enforce layer specific control. Naka A, Veit J, Shababo B, Chance RK, Risso D, Stafford D, Snyder B, Egladyous A, Chu D, Sridharan S, Mossing DP, Paninski L, Ngai J, Adesnik H. *eLife*. 2019 Mar 18;8. pii: e43696. doi: 10.7554/eLife.43696.
 - c) Superficial Layers Suppress the Deep Layers to Fine-tune Cortical Coding. Pluta SR, Telian GI, Naka A, Adesnik H. *J Neuroscience*. 2019 Mar 13;39(11):2052-2064. doi: 10.1523/JNEUROSCI.1459-18.2018. Epub 2019 Jan 16. PMID: 30651326
 - d) Layer-specific excitation/inhibition balances during neuronal synchronization in the visual cortex. Adesnik H. *J Physiology* 2018 May 1;596(9):1639-1657. doi: 10.1113/JP274986. Epub 2018 Jan 24. PMID: 29313982
3. **Novel optical and genetic tools to interrogate the nervous system:** This collection of papers outlines my efforts to establish new optical and optogenetic approaches to probe the inner workings of neural circuits. Much of our effort has been directed at developing an all-optical multiphoton holographic optogenetic approach for controlling neural activity in space and time with very high precision. I have also helped develop and validate novel photochemical and optogenetic tools to probe the operation of specific neuronal subtypes in the mammalian central nervous system. These latter tools have opened up optical manipulation of cellular and circuit function at the molecular level.
 - a) Precise multimodal optical control of neural ensemble activity. Mardinly AR, Oldenburg IA, Pegard NC, Sridharan S, Waller L, Adesnik H. *Nature Neuroscience* 2018 Jun;21(6):881-893. doi:

- 10.1038/s41593-018-0139-8.
- b) 3D scanless holographic optogenetics with temporal focusing. Pegard NC, Mardinly AR, Oldenburg IA, Sridharan S, Waller L, Adesnik H. *Nature Communications* Oct 31;8(1):1228. doi: 10.1038/s41467-017-01031-3. PMID: 29089483
 - c) A comprehensive optogenetic toolkit for in vivo control of endogenous GABA_A receptors and synaptic inhibition. Lin, W., Tsai, M., Davenport C., Smith, C., Wilson, N., Adesnik, H., Kramer, R.H. *Neuron* 2015 Dec 2;88(5):879-91 PMID: 26606997
 - d) Three-dimensional Multi-site Random Access Photostimulation (3D-MAP) Yi Xue, Laura Waller, Hillel Adesnik, Nicolas Pégard bioRxiv 2020.06.28.176503; doi: <https://doi.org/10.1101/2020.06.28.176503>
- 4. Synaptic and circuit basis of touch perception.** This work investigates the cortical circuits responsible for encoding and processing somatosensory information in the rodent whisker system. Combining multi-electrode array electrophysiology, optogenetics, and intracellular recording *in vivo* and *in vitro*, I have revealed microcircuits in the somatosensory cortex that are responsible for encoding elementary features of tactile objects.
- a) Spatial integration during active tactile sensation drives elementary shape perception Jennifer Brown, Ian Antón Oldenburg, Gregory I. Telian, Sandon Griffin, Mieke Voges, Vedant Jain, Hillel Adesnik *Neuron* In press
 - b) Surround integration organizes a spatial map during activations sensation. Pluta SR, Lyall EH, Telian GI, Ryapolova-Webb E, Adesnik H. *Neuron* 2017 Jun 21;94(6):1220-1233.e5. PMCID: PMC5512457A
 - c) A direct translaminar inhibitory circuit tunes cortical output. Pluta, S., Naka, A., Veit, J., Telian, G., Hakim, R., Adesnik, H. *Nature Neuroscience* 2015 Nov;18(11):1631-40 PMCID: PMC4624464
 - d) Adesnik, H. and M. Scanziani (2010) "Lateral competition for cortical space by layer-specific horizontal circuits." *Nature* 464(7292): 1155-60. PMCID: PMC2908490
- 5. Inhibitory synapses and circuitry in the primary visual cortex:** These papers address the development, wiring, and function of inhibitory circuits in the neocortex. They combine sophisticated structured illumination optogenetics or cell type specific manipulation to ask how specific inhibitory pathways of subtypes in the mouse's primary visual cortex shape the stimulus driven activity of excitatory neuronal populations, with a specific focus on lateral inhibition through horizontal circuitry, and gain control through vertical circuitry.
- a) A neural circuit for gamma-band coherence across the retinotopic map in mouse visual cortex. Hakim R, Shamardani K, Adesnik H. *Elife*. 2018 Feb 26;7. PMID: 29480803
 - b) Sensory experience regulates cortical inhibition by inducing IGF1 in VIP neurons. Mardinly AR, Spiegel I, Patrizi A, Centofante E, Bazinet JE, Tzeng CP, Mandel-Brehm C, Harmin DA, Adesnik H, Fagiolini M, Greenberg ME. *Nature*. 2016 Mar 17;531(7594):371-5. PMCID: MC4823817
 - c) Input normalization by global feedforward inhibition expands cortical dynamic range. Pouille F, Marin-Burgin A, Adesnik H, Atallah BV, Scanziani M. *Nat Neurosci*. 2009 Dec;12(12):1577-85. PMID: 19881502
- 6. Molecular mechanisms governing synaptic strength:** These papers probed the cell biological regulation of synaptic strength. I helped discover accessory proteins that modulate both trafficking and gating of AMPA receptors, secreted factors that control AMPA receptor number at the synapse, the time course of AMPA receptor insertion to synapses, and the critical role of NMDA-type glutamate receptors in controlling synaptic AMPA receptor abundance.
- a) Adesnik, H., G. Li, et al. (2008). "NMDA receptors inhibit synapse unsilencing during brain development." *Proc Natl Acad Sci U S A* 105(14): 5597-602. PMID: 18375768
 - b) Adesnik, H. and R. A. Nicoll (2007). "Conservation of glutamate receptor 2-containing AMPA receptors during long-term potentiation." *J Neurosci* 27(17): 4598-602. PMID: 17460072
 - c) Fukata, Y., H. Adesnik, et al. (2006). "Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission." *Science* 313(5794): 1792-5. PMID: 16990550
 - d) Tomita, S., H. Adesnik, et al. (2005). "Stargazin modulates AMPA receptor gating and trafficking by distinct domains." *Nature* 435(7045): 1052-8. PMID: 15858532

D. Research Support

Ongoing Research Support

R01 EY023756-01 (PI)

09/01/2018-08/31/2023

NIH/NEI

Excitation and Inhibition in Neural Circuits of the Visual Cortex

The goal of this study is to determine how the excitation/inhibition balance across layers of the visual cortex subserves visual computation.

Role: PI

UF1NS107574 (PI)

09/30/2018-08/31/2021

NINDS

High speed, high precision volumetric multiphoton neural control

The goal of the project is to optimize and expand the capabilities of multiphoton holographic optogenetics for controlling precise neural ensembles with light in multiple species and in deep brain structures.

RF1MH120680 (PI)

08/01/2019-05/31/2022

High-throughput Physiological Micro-connectivity Mapping in Vivo

The goal of this project is to develop optical and electrophysiological tools to comprehensively map local and long-range monosynaptic connectivity in the brain.

U19NS107613 (PI: Ken Miller)

09/15/2018-06/30/2023

NINDS

Understanding V1 circuit dynamics and computations

The goal of this project is to combine theory with experiment to understand neural computation in the visual cortex.

Completed Research Support

R01 EY023756-01 (PI)

09/01/2013-08/31/2018

NIH/NEI

Excitation and Inhibition in Neural Circuits of the Visual Cortex

The goal of this study is to determine how the excitation/inhibition balance across layers of the visual cortex subserves visual computation.

Role: PI

NYSCF-R-N136 (PI)

01/01/2016-12/31/2020

New York Stem Cell Foundation

Cracking the neural codes of sensory perception

The major focus of this project is to employ multiphoton optogenetics to bi-directionally edit the neural ensembles of sensory perception in real time during behavior.

Role: PI

1DP2NS087725-01

09/30/2013-07/31/2018

NIH/NINDS

New Optical Strategies to Unlock the Neural Basis of Perception

The goal of this study is to develop new approaches to use light to dissect the neural circuits for sensory perception.

Role: PI

BIOGRAPHICAL SKETCH

DO NOT EXCEED FIVE PAGES.

NAME: Ji, Na

eRA COMMONS USER NAME (credential, e.g., agency login): simplive

POSITION TITLE: Associate Professor of Physics and Molecular Cell Biology

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Science and Technology of China, Hefei, Anhui, P. R. China	B.S.	06/2000	Chemical Physics
University of California, Berkeley, CA, United States	Ph.D.	12/2005	Chemistry and Physics
University of California, Berkeley, CA, United States	Postdoctoral	10/2006	Physics
Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, United States	Postdoctoral	03/2011	Physics and Neurobiology

A. Personal Statement

I have been a faculty member at the Molecular and Cell Biology (MCB) Department at UC Berkeley since 2016. Prior to UC Berkeley, I was a group leader at Janelia Research Campus, Howard Hughes Medical Institute (HHMI). My research centers on developing novel imaging methods and applying them to understand neural circuits. To understand neural circuits, we need to monitor the activity of neurons distributed millimeters in area and depth but with sub-micron spatial and millisecond temporal resolution. Since 2011, my laboratory has used concepts in astronomy and optics, such as adaptive optics and wavefront engineering, to develop next-generation microscopy methods for imaging the brain at higher resolution, greater depth, and faster speed. We have achieved synapse-level spatial resolution throughout the entire depth of primary visual cortex, optimized microendoscopes for imaging deeply buried nuclei, developed video-rate (30 Hz) volumetric imaging and kilohertz frame-rate voltage imaging methods. We apply these methods to understanding neural circuits in early visual pathway, using mouse as our model system. We actively work on disseminating our imaging methods, so that the entire neurobiological community can benefit from our work. Throughout my career, I have worked and thrived at the interfaces of sciences. I enjoy speaking the languages of different scientific disciplines and have witnessed repeatedly how interdisciplinary research has transformed chemistry, physics, and biology. I have collaborated extensively with researchers from other institutions (e.g., HHMI, Purdue University, NIDA, UC Davis, Max Planck Florida Institute, Champalimaud, Albert Einstein College of Medicine) and produced peer-reviewed publications for all collaborative projects. I have existing collaborations with Dr. Adesnik, with members of our laboratories working together on several projects. For this proposal, we will utilize our expertise on kilohertz full-frame two-photon fluorescence microscopy imaging of membrane voltage *in vivo* to test ASAP variants and validate holographic two-photon imaging results.

- a. Wang, C., Liu, R., Milkie, D. E., Sun, W., Tan, Z., Kerlin, A., Chen, T. W., Kim, D. S. & Ji, N. (2014) Multiplexed aberration measurement for deep tissue imaging *in vivo*. **Nature Methods**, 11(10), 1037-1040.
- b. Wang, K., Sun, W., Richie, C. T., Harvey B. K., Betzig, E. & Ji, N. (2015) Direct wavefront sensing for high-resolution *in vivo* imaging in scattering tissue. **Nature Communications**, 6, 7276.
- c. Lu, R., Sun, W., Liang, Y., Kerlin, A., Bierfeld, J., Seelig, J., Wilson, D. E., Scholl, B., Mohar, B., Tanimoto, M., Koyama, M., Fitzpatrick, D., Orger, M. B., Ji, N. (2017) Video-rate volumetric functional imaging of the brain at synaptic resolution, **Nature Neuroscience**, 20, 620-628.
- d. Lu, R., Liang, Y., Meng, G., Zhou, P., Svoboda, K., Paninski, L., Ji, N. (2020) Rapid mesoscale volumetric imaging of neural activity with synaptic resolution. **Nature Methods** 17, 291-294.

- e. Wu, J., Liang, Y., Chen, S., Hsu, C.-L., Chavarha, M., Evans, S.W., Shi, D., Lin, M. Z., Tsia, K. K., Ji, N. (2020) Kilohertz two-photon fluorescence microscopy imaging of neural activity *in vivo*. **Nature Methods** 17, 287-290.

B. Positions and Honors

Positions and Employment

- 2011-2017 Group Leader, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA
 2016- Associate Professor, Department of Molecular Cell Biology and Department of Physics, University of California, Berkeley, CA

Other Experience and Professional Memberships

- 2008- Member, Society for Neuroscience
 2011- Member, Optical Society of America
 2015- Associate Editor, Biomedical Optics Express

Honors

- 2016 Cornelis Wiersma Visiting Professor, California Institute of Technology, CA
 2017-2022 Luis Alvarez Memorial Chair in Experimental Physics, UC Berkeley, CA

C. Contribution to Science

1. **High-speed brain imaging.** A performance metric for *in vivo* brain imaging that needs improvement is the volumetric imaging speed, which is limited by the serial focal scanning of two-photon microscopy and the limited brightness of indicators. We designed an optical module that can be easily integrated into standard 2PLSMs to generate an axially elongated Bessel focus. Scanning the Bessel focus in 2D turned frame rate into volume rate and enabled video-rate (30 Hz) volumetric imaging. Using Bessel foci designed to maintain synaptic-level lateral resolution *in vivo*, we demonstrated the power of this approach in enabling discoveries for neurobiology by imaging the calcium dynamics of volumes of neurons and synapses in fruit flies, zebrafish larvae, mice, and ferrets *in vivo*. We are developing this method further to monitor the calcium activity dynamics of all neurons in an entire cortical circuit. We have also developed a kilohertz two-photon fluorescence microscope and used it to image both subthreshold and suprathreshold membrane voltage events.

- a. Lu, R., Sun, W., Liang, Y., Kerlin, A., Bierfeld, J., Seelig, J., Wilson, D. E., Scholl, B., Mohar, B., Tanimoto, M., Koyama, M., Fitzpatrick, D., Orger, M. B., Ji, N. (2017) Video-rate volumetric functional imaging of the brain at synaptic resolution, **Nature Neuroscience**, 20, 620-628.
- b. Lu, R., Liang, Y., Meng, G., Zhou, P., Svoboda, K., Paninski, L., Ji, N. (2020) Rapid mesoscale volumetric imaging of neural activity with synaptic resolution. **Nature Methods** 17, 291-294.
- c. Wu, J., Liang, Y., Chen, S., Hsu, C.-L., Chavarha, M., Evans, S.W., Shi, D., Lin, M. Z., Tsia, K. K., Ji, N. (2020) Kilohertz two-photon fluorescence microscopy imaging of neural activity *in vivo*. **Nature Methods** 17, 287-290.

2. **High-resolution brain imaging.** In microscopy, when light travels through biological samples, its wavefront is distorted by tissue refractive-index variations. As a result of these sample-induced optical aberrations, image resolution and signal are compromised, and diffraction-limited imaging performance is rarely achieved in live tissue. Canceling out these aberrations with active shaping of light wavefront, one can recover ideal resolution and signal. First working as a postdoc in the laboratory of Eric Betzig, and later in my own laboratory, I have pioneered the development and application of adaptive optics methods for aberration correction in live brains *in vivo*. We achieved large field-of-view, diffraction-limited imaging throughout cortices of live mice, substantially extending the achievable depth for high-resolution brain imaging.

- a. Ji, N., Milkie D. E. & Betzig, E. (2009) Adaptive optics via pupil segmentation for high-resolution imaging in biological tissues. **Nature Methods**, 7(2), 141-147.
- b. Ji, N., Sato, T. R. & Betzig, E. (2012) Characterization and adaptive optical correction of aberrations during *in vivo* imaging in the mouse cortex. **Proceedings of the National Academy of Sciences**, 109(1), 22-27.

- c. Wang, C., Liu, R., Milkie, D. E., Sun, W., Tan, Z., Kerlin, A., Chen, T. W., Kim, D. S. & Ji, N. (2014) Multiplexed aberration measurement for deep tissue imaging *in vivo*. **Nature Methods**, 11(10), 1037-1040.
 - d. Wang, K., Sun, W., Richie, C. T., Harvey B. K., Betzig, E. & Ji, N. (2015) Direct wavefront sensing for high-resolution *in vivo* imaging in scattering tissue. **Nature Communications**, 6, 7276.
3. **Deep brain imaging.** In addition to aberration, *in vivo* imaging depth in opaque samples such as mouse brain is limited by light scattering. To image deeply buried nuclei *in vivo*, one method is to use miniature endoscopy probes that are embedded in the brain for image relay. There are two problems associated with this approach: the compromised image quality by the intrinsic aberration of the probes and the damage caused by probe implantation. My laboratory systematically characterized the optical performance of endoscopy probes and developed methods to improve image quality and enlarge image field of view. Using off-the-shelf components, my laboratory also developed a minimally invasive endoscopy system and, in collaboration with two other laboratories, used it to observe neural activity from deeply buried nuclei such as lateral hypothalamus and substantia nigra with subcellular resolution in head-fixed awake mice.
- a. Wang, C. & Ji, N. (2012) Pupil-segmentation-based adaptive optical correction of a high-numerical-aperture gradient refractive index lens for two-photon fluorescence endoscopy. **Optics Letters**, 37(11), 2001-2003.
 - b. Wang, C. & Ji, N. (2013) Characterization and improvement of three-dimensional imaging performance of GRIN-lens-based two-photon fluorescence endomicroscopes with adaptive optics. **Optics Express**, 21(22), 27142-27154
 - c. Bocarsly, M. E., Jiang, W.-C., Wang, C., Dudman, J. T., Ji, N. & Aponte, Y. (2015) A minimally invasive microendoscopy system for *in vivo* functional imaging of deep nuclei in the mouse brain. **Biomedical Optics Express**, 6(11), 4546-4556.
 - d. Meng, G., Liang, Y., Sarsfield, S., Jiang, W. C., Lu, R., Dudman, J. T., Aponte, Y., Ji, N. (2019) High-throughput synapse-resolving two-photon fluorescence microendoscopy for deep-brain volumetric imaging *in vivo*. **Elife**, e40805.
4. **In vivo testing genetically encoded fluorescent sensors.** Fluorescence microscopy relies on fluorescent sensors for providing information on the morphology and function of biological structures. I have collaborated with multiple groups in testing the performance of fluorescent sensors in the context of brain imaging.
- a. Richie, C. T., Whitaker, L. R., Whitaker, K. W., Necarsulmer, J., Baldwin, H. A., Zhang, Y., Fortuno, L., Hinkle, J. J., Koivula, P., Henderson, M. J., Sun, W., Wang, K., Smith, J. C., Pickel, J., Ji, N., Hope, B. T., Harvey, B. K. (2017) Near-infrared fluorescent protein iRFP713 as a reporter protein for optogenetic vectors, a transgenic Cre-reporter rat, and other neuronal studies. **Journal of Neuroscience Methods** 284, 1-14.
 - b. Grimm, J. B., Muthusamy, A. K., Liang, Y., Brown, T. A., Lemon, W. C., Patel, R., Lu, R., Macklin, J. J., Keller, P. J., Ji, N., Lavis, L. D. (2017) A general method to fine-tune fluorophores for live-cell and *in vivo* imaging. **Nature Methods** 14, 987-994.
 - c. Broussard, G., Liang, Y., Fridman, M., Unger, E., Meng, G., Xiao, X., Ji, N., Petreanu, L., Tian, L. (2018) *In vivo* measurement of afferent activity with axon-specific calcium imaging. **Nature Neuroscience** 21, 1272-1280.
 - d. Shemetov A. A., Monakhov M. V., Zhang Q., Canton-Josh J. E., Kumar M., Chen M., Matlashov M. M., Li X., Yang W., Nie L., Shcherbakova D. M., Kozorovitskiy Y., Yao J., Ji N., Verkhusha V. V. (2020) Bright near-infrared genetically encoded calcium indicator for *in vivo* imaging. **Nature Biotechnology** doi: 10.1038/s41587-020-0710-1
5. **Application of imaging methods to study visual circuits.** My laboratory applies the advanced imaging technologies to the study of circuit computation, using mouse primary visual cortex (V1) as a model system. Like most mammals, the mouse retina contains two cone opsins, but with the short-wavelength opsin absorbing ultraviolet light. The function of UV vision in the mostly nocturnal mice was not known, partly because the neural representation of UV vision in mouse central nervous system had never been investigated. We studied the response of mouse V1 to UV visual stimuli and discovered that mouse V1

encodes UV visual information very similarly to how it encodes stimuli in the visible spectrum, suggesting that UV vision allows the mouse to broaden the spectral range of visual perception. Having characterized the full spectral inputs of mouse visual system, we turned our attention to the circuit mechanism of orientation selectivity. Since Hubel and Wiesel's pioneering work, the computation underlying the orientation selectivity of cortical neurons in V1 has been a flagship question in neuroscience. The dominant view has been that orientation selectivity results from thalamocortical interaction in V1, with the thalamic inputs themselves lacking orientation selectivity. With the help of adaptive optics, we directly measured the tuning property of thalamic boutons in layer 4 of mouse V1, and found that around half of the boutons are already orientation-tuned. These tuned inputs have strong directional biases that are conserved in the selectivity of the cortical output L5 neurons. We provided the first demonstration where functional properties of boutons were examined deep inside cortex, as well as the first experimental evidence that thalamus provides V1 with substantial orientation-tuned input. Our experimental approach, where the representational/tuning properties of thalamic boutons and cortical neurons in the input and output layers of a neural circuit are systematically characterized, can be broadly applied as a data-rich method to elucidate circuit computation.

- a. Tan, Z., Sun, W., Chen, T., Kim, D. & Ji, N. (2015) Neuronal representation of ultraviolet visual stimuli in mouse primary visual cortex. *Scientific Reports*, 5, 12597.
- b. Sun, W., Tan, Z., Mensh, B. & Ji, N. (2016) Thalamus provides layer 4 of primary visual cortex with orientation- and direction-tuned inputs. *Nature Neuroscience*, 19, 308-315.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/48914354/?sort=date&direction=descending>

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1U01NS103489 (PI: Ji)	07/15/2017 – 06/30/2020 NCE
High-speed volumetric imaging of neural activity throughout the living brain	
The goal of this project is to leverage recent advances in imaging technologies and data sciences to enable high-speed, high-resolution, and high-throughput volumetric imaging of neural circuit activity in vivo.	
Role: Co-investigator	
1U01NS103573 (PI: Verkhusha)	08/01/2017 – 07/31/2020 NCE
Calcium biosensors for deep-tissue imaging and spectral multiplexing	
We will characterize near-infrared sensors based on iRFP for non-invasive deep-tissue imaging in mouse brain in vivo.	
Role: Co-investigator	
1U01NS103571 (PI: Zito)	06/01/2017 - 05/31/2020 NCE
Large-scale analysis of functional synapses during circuit plasticity with novel optogenetic sensors	
We will characterize novel glutamate sensors for large-scale monitoring of synaptic activity in mouse brain in vivo.	
Role: Co-investigator	
2R44MH111463-02A1 (PI: Linnenberger)	05/01/2018 – 04/30/2019 NCE
An add-on microscopy module for fast volumetric imaging	
The goal of this project is to test the high-speed, high-resolution spatial light modulator that will be developed in the phase II by Meadowlark Optics, and validate the volume accessible and imaging rate in biological preparations.	
Role: Co-investigator	
1UF1NS107696 (PI: Ji)	09/01/2018 – 08/31/2021
kHz-rate in Vivo Imaging of Neural Activity throughout the Living Brain	
The goal of this project is to utilize a novel optical scanning strategy to achieve kHz-rate in vivo imaging of neural activity.	
Role: PI	

1U19NS107613	(PI: Miller)	07/01/2018 – 06/30/2023
Understanding V1 circuit dynamics and computations		
Subproject title: Cell-type specific characterization of neuronal activity throughout V1		
The goal of this project is to obtain <i>in vivo</i> neural imaging data to constrain and validate theoretical models of mouse primary visual cortex during behavior.		
Role: Co-investigator		
UF1NS107574	(PI: Adesnik)	09/30/2018 - 08/31/2021
High speed, high precision volumetric multiphoton neural control		
The goal of the project is to optimize and expand the capabilities of multiphoton holographic optogenetics, increasing the addressable volume and number of neurons that can be simultaneously photo-stimulated, and combining with Bessel beam imaging and micro-endoscopy for accessing deep brain structures.		
Role: Co-investigator		
UCB 20182438	(PI: Halgren)	09/01/2019 – 08/31/2024
NIH		
Integrated Biophysical and Neural Model for Cortical Effects of Electrical Stimulation		
The goal of the project is to develop and test biophysical models of how electric stimulation affects cortical Activity.		
Role: Co-investigator		
1RF1MH120680	(PI: Adesnik)	09/01/2019 – 08/31/2022
NIH/BRAIN Initiative		
High-throughput physiological micro-connectivity mapping <i>in vivo</i>		
The goal is to develop analysis techniques and closed-loop Bayesian optimal experimental design methods for estimating synaptic connectivity and plasticity models.		
Role: Co-investigator		
Neurohub		04/01/2020 – 05/01/2021
Weill Neurohub Pillar Project Award		
Next-Generation Microscopic Imaging Platforms for Live Brain Imaging		
A collaboration grant with UCSF and University of Washington on applying advanced microscopy imaging platform for live brain imaging of <i>C elegans</i> , tardigrade, and hydra.		
Role: Co-investigator		
1U01NS118300		01/15/2021 – 12/31/2023
NIH-NINDS		
Adaptive Optical Microscopy for High-Accuracy Recording of Neural Activity in Vivo		
The goal is to combine adaptive optics with the leading brain imaging modalities to achieve high-accuracy <i>in vivo</i> functional imaging of neural activity throughout the living brain.		

Completed Research Support

None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Brohawn, Stephen Graf

eRA COMMONS USER NAME (credential, e.g., agency login): sbrohawn

POSITION TITLE: Assistant Professor of Neurobiology

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Delaware	B.S.	05/2004	Biochemistry, Chemistry
Massachusetts Institute of Technology	Ph.D.	05/2010	Structural Biology
The Rockefeller University	Postdoctoral	12/2015	Neuroscience

A. Personal Statement

My laboratory studies the molecular and mechanistic basis of cellular electrical signaling. I have an extensive background in molecular neuroscience and biophysics including specific training in structural, electrophysiological, and imaging approaches to study ion channels and transporters. My laboratory has demonstrated expertise in cryo-EM and X-ray crystallography, electrophysiology, biochemistry, fluorescence imaging, and molecular tool development. My work as a graduate student provided structural and biophysical insight into the evolution and functional organization of the nuclear pore complex (NPC). My work as a postdoctoral fellow revealed the physical basis for mechanosensitivity of two-pore domain potassium (K2P) channels and demonstrated these channels are localized to nodes of Ranvier in myelinated axons. As an independent investigator, my lab has published work on the structure and function of mechanosensitive K2P channels, pH-gated K2P channels, volume-regulated KCC transporters, and volume-regulated anion channels. In addition, we have published work related to the development of molecular tools for optogenetics and sonogenetics. Specifically related to this proposal, we have collaborated with the Adesnik lab on the structure-based design of two iterations of improved opsins called ChroME and ChroME2f/s based on the starting opsin Chronos. Our role in these projects was to model opsin structures, predict mutations that would improve opsin properties, and to contribute to the characterization of mutant opsins in cultured cells. The work in this proposal is a direct extension of these efforts. In addition, my lab has made technical advances for high resolution structure determination of small membrane proteins by cryo-EM. We will apply these advances to opsins in order to understand mechanistic impacts of opsin modification through an iterative process of design, functional evaluation, and structure determination.

B. Positions and Honors

Positions and Employment

- 2004-2010 Ph.D. student, Massachusetts Institute of Technology, Laboratory of Thomas Schwartz
2010-2015 Postdoctoral fellow, HHMI, Rockefeller University, Laboratory of Roderick MacKinnon
2016-current Assistant Professor of Neurobiology, University of California, Berkeley

Honors

- 2002-2004 Beckman Scholar Undergraduate Fellowship
2003 HHMI Research Undergraduate Fellowship
2004 University of Delaware Honors Degree with Distinction
2004 Elizabeth Dyer Award for Excellence in Biochemistry and Chemistry
2004 Frank W. Collins Undergraduate Award in Biochemistry
2004 Phi Beta Kappa

2007-2008	Vertex Scholar Graduate Student Fellowship
2008-2009	Koch Institute Graduate Student Fellowship
2011-2014	Helen Hay Whitney Postdoctoral Fellowship
2011	Damon Runyon Postdoctoral Fellowship (declined)
2014	Blavatnik Award for Young Scientists Finalist
2014	Regeneron Pharmaceuticals Prize for Creative Innovation Finalist
2016-2019	Klingenstein-Simons Fellowship Award in the Neurosciences
2016-2021	NIH Director's New Innovator Award
2017-2020	McKnight Neuroscience Scholar Award
2017-2022	New York Stem Cell Foundation Robertson Neuroscience Investigator Award
2019-2021	Rose Hill Innovator Award (with H. Adesnik, UC Berkeley)
2020-2021	FastGrant Award for Covid19 Research (with H. Adesnik and D. Bautista and, UCB)
2020-2022	Rennie Fund for the Study of Epilepsy Award (with R. Kramer, UCB)
2020	American Association for Anatomy R.R. Bensley Award in Cell Biology
2020-2022	Alfred P. Sloan Research Fellowship
2021	Winkler Scholar Faculty Award

C. Contribution to Science

- Nuclear pore complex structure and function:** My work as a graduate student provided insight into the evolution and functional organization of the nuclear pore complex (NPC). NPCs perforate the nuclear membrane to form conduits for transport of molecules between the nucleus and cytoplasm. NPCs are among the cell's largest molecular machines and are challenging to study due to their size and complexity. I determined three protein complex crystal structures to construct a model of a half-megadalton subcomplex of the NPC structural scaffold. These structures revealed that proteins in the NPC scaffold adopt a unique helical fold only previously observed in COPII vesicle coat proteins, providing structural evidence of an evolutionary relationship between the NPC and COPII coat. Based on this relationship, a common protein arrangement in the NPC and COPII coat was predicted and verified with structural, biophysical and genetic experiments. This led to a model in which the NPC scaffold functions to stabilize nuclear membrane curvature analogous to how the COPII coat stabilizes vesicle membrane curvature.

 - a) Brohawn, SG*, Leksa, NC*, Spear, ED, Rajashankar, K & Schwartz, TU. Structural evidence for common ancestry of the nuclear pore complex and vesicle coats. *Science* 322, 1369–1373 (2008). PMCID: PMC2680690
 - b) Leksa, NC*, Brohawn, SG* & Schwartz, TU. The structure of the scaffold nucleoporin Nup120 reveals a new and unexpected domain architecture. *Structure* 17, 1082–1091 (2009). PMCID: PMC2743489
 - c) Brohawn, SG*, Partridge JR*, Whittle, JRR* & Schwartz, TU. The nuclear pore complex has entered the atomic age. *Structure* 17, 1156–1168 (2009). PMCID: PMC2776643
 - d) Brohawn, SG & Schwartz, TU. Molecular architecture of the Nup84-Nup145C-Sec13 edge element in the nuclear pore complex lattice. *Nature Structure and Molecular Biology* 16, 1173–1177 (2009). PMCID: PMC3398507
- Structure, function, and physiology of the mechanosensitive K⁺ channel TRAAK:** These studies were aimed at understanding how ion channels are activated by mechanical force at structural, mechanistic, and physiological levels. They represent one of our most complete understandings of eukaryotic mechanosensation by ion channels to date. I used reductionist assays to show that TRAAK and related K2P channels are membrane tension-gated, extending the force-from-lipid principle discovered in unrelated bacterial channels to animals. I solved a series of crystal structures of human TRAAK in multiple conformations and with corroborating electrophysiological data derived a model for the physical basis of channel gating and mechanosensitivity. Our model for gating of TRAAK channels involved unprecedented lipid block of ion permeation in the nonconductive state. Opening the channel involves sealing holes to the membrane to prevent lipid block. Shape changes including area expansion upon opening provide a biophysical explanation for channel mechanosensitivity. Finally, I determined

TRAALK is specifically localized to nodes of Ranvier in myelinated axons, suggesting an unappreciated mechanical aspect of saltatory conduction.

- a) Brohawn, SG, del Mármlol, J & MacKinnon, R. Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K⁺ ion channel. *Science* 335, 436–441 (2012). PMCID: PMC3329120
 - b) Brohawn, SG, Campbell, EB & MacKinnon, R. Physical mechanism for gating and mechanosensitivity of the human TRAAK K⁺ channel. *Nature* 516 (7529), 126-30 (2014). PMCID: PMC4682367
 - c) Brohawn, SG, Wang, W, Schwarz, JR, Handler, A, Campbell, EB & MacKinnon, R. The Mechanosensitive ion channel TRAAK is localized to the mammalian node of Ranvier. *eLife*;8:e50403 (2019); bioRxiv 713990. PMCID: PMC6824864
 - d) Rietmeijer, RA[#], Sorum, B[#], Li, B & Brohawn SG. The mechanistic basis for distinct leak and mechanically gated activity of the human two-pore domain K⁺ channel TRAAK. *Neuron* (in revision).
- 3. Mechanisms of gating and regulation of K2P channels:** We are investigating mechanisms of gating and chemical regulation of leak-type K⁺ channels. The first paper from these efforts project presents cryo-EM structures and functional studies that reveal how the TASK2 K⁺ channel is gated by pH by distinct intracellular and extracellular gates.
- a) Li, B, Rietmeijer, RA & Brohawn, SG. Structural basis for pH-gating of the two-pore domain K⁺ channel TASK2. *Nature*. 586(7829):457-462 (2020); PMID: 32999458
- 4. Mechanisms of cellular volume regulation.** My lab has studied cellular volume regulation by ion channels and transporters. One project is focused on understanding principles that underlie the function and regulation of volume-regulated anion channels (VRACs) formed by LRRC8s. Structures of LRRC8A in lipid nanodiscs determined in the presence and absence of the blocker DCPIB by cryo-EM revealed the architecture of LRRC8A in a lipid environment, the mechanism for block by a small molecule drug, and conformational changes that we propose are correlated with gating conformational changes. A second project is focused on understanding the basis for the function and regulation of potassium-chloride cotransporters (KCCs). The structure of KCC4 in lipid nanodiscs determined by cryo-EM revealed the architecture of KCC4 including a novel extracellular domain that forms an outer gate, the basis for differences in substrate stoichiometry and selectivity among related cation-chloride cotransporters, and insights into regulation of transport activity by osmotic stimuli.
- a) Kern, DM, Oh, S, Hite, RK & Brohawn, SG. Cryo-EM structures of the DCPIB-inhibited volume-regulated anion channel LRRC8A in lipid nanodiscs *eLife* 2019;8:e42636 DOI: 10.7554/eLife.42636 PMCID: PMC6395065
 - b) Reid, MS, Kern, DM & Brohawn, SG. Cryo-EM structure of the potassium-chloride cotransporter KCC4 in lipid nanodiscs. *eLife* 9:e52505 (2020); PMCID: PMC7200160
 - c) Gunasekar, SK, Xie, L, Chheda, PR, Kang, C, Kern, DM, My-Ta, C, Kumar, A, Maurer, J, Gerber, EE, Grzesik, WJ, Elliot-Hudson, M, Zhang, Y, Kulkarni, CA, Samuel, I, Smith, JK, Nau, P, Imai, Y, Sheldon, RD, Taylor, EB, Lerner, DJ, Norris, AW, Brohawn, SG, Kerns, R & Sah, R. Small molecule SWELL1-LRRC8 complex induction improves glycemic control and nonalcoholic fatty liver disease in murine Type 2 diabetes. *bioRxiv* 2021.02.28.432901; doi: <https://doi.org/10.1101/2021.02.28.432901>
- 5. Development channel-based tools for neuromodulation:** We collaborate with Prof. H. Adesnik at UC Berkeley to develop and characterize molecular tools for neuromodulation. The first effort is to generate variants of light-gated channels (channelrhodopsins) with improved properties for multiphoton, large volume neural modulation. We have used a combination of structure-based mutagenesis and electrophysiology to iteratively generate opsins with improved kinetic properties and increased photocurrents. The second effort is to understand the mechanistic basis for ultrasonic neuromodulation and to develop genetically targetable, ultrasound activated ion channels as tools for “sonogenetics”. We have shown low intensity, low frequency ultrasound rapidly and robustly activates TRAAK channels in a manner analogous to canonical mechanical activation: by generating tension in the membrane to favor channel opening.
- a) Mardirily, AR, Oldenburg, IA, Pégard, NC, Sridharan, S, Lyalkl, E, Chesnov, K, Brohawn, SG, Waller, L & Adesnik, H. Precise bidirectional spatiotemporal control of neural activity. *Nature Neuroscience*. 21(6):881-893 doi: 10.1038/s41593-018-0139-8. Epub 2018 Apr 30. (2018) PMID: 29620000

- 29713079; PMCID: PMC5970968
- b) Sorum, B, Rietmeijer, RA, Gopakumar, K, Adesnik, H & Brohawn, Brohawn, SG. Ultrasound activates mechanosensitive TRAAK K⁺ channels directly through the lipid membrane. *bioRxiv* 2020.10.24.349738. doi: <https://doi.org/10.1101/2020.10.24.349738>. *Proceedings of the National Academy of Sciences*, 118(6):e2006980118. doi: 10.1073/pnas.2006980118. (2020). PMID: 33542098.
 - c) Sridharan, S, Gajowa, M, Ogando, MB, Jagadisan, U, Ahdeladim, L, Sadahiro, M, Bounds, H, Hendricks, HD, Tayler, I, Gopakumar, K, Oldenburg, IA, Brohawn, SG, Adesnik, HA. *bioRxiv* 2021.04.01.438134; doi: <https://doi.org/10.1101/2021.04.01.438134> (2021).

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40298401/?sort=date&direction=descending>

D. Research Support

Ongoing Research Support

DP2 GM123496 NIH Director's New Innovator Award (Brohawn) 09/30/2016 - 06/30/2021
NIH/NIGMS

"New Approaches to Understanding Biological Force Sensation"

The goal of this project is to develop new approaches including pharmacological and optical tools to elucidate principles of force sensation.

Role: PI

McKnight Neuroscience Scholar Award (Brohawn) 07/01/2017- 06/30/2021

McKnight Endowment Fund for Neuroscience

"Mechanisms of Biological Force Sensation"

The goal of this project is to determine the molecular basis of hearing and balance through structural and mechanistic studies of proteins involved in force transduction.

Role: PI

New York Stem Cell Robertson Neuroscience Investigator Award (Brohawn) 01/01/2018 - 12/31/2022

New York Stem Cell Foundation

"Principles of Force Sensation in the Nervous System"

This is a career development award not tied to a specific project and will support all research in the lab.

Role: PI

UF1 NIH NINDS Brohawn (co-PI with Adesnik, H.) 09/30/2018 - 08/31/2021
NIH/NINDS

"High speed, high precision volumetric multiphoton neural control"

Our role in the multi-PI project is to rationally engineer improved opsins for fast neuromodulation.

Role: Co-Investigator

Rose Hill Innovator Award Brohawn (co-PI with Adesnik, H.) 07/01/2019 - 06/30/2021
UC Berkeley

"Development of a sonogenetic toolkit to control neural activity with ultrasound"

The goal of this joint project with Dr. Adesnik's lab is to develop a set of ultrasound activated ion channels to control neural activity.

Role: PI

Alfred P. Sloan Research Fellowship Award in Neuroscience (Brohawn) 01/01/2020 – 01/01/2022
Alfred P. Sloan Foundation

This is a career development award not tied to a specific project and will support all research in the lab.

Role: PI

BIOGRAPHICAL SKETCH

NAME: Waller, Laura

ERA COMMONS USER NAME: Waller

POSITION TITLE: Associate Professor of Electrical Engineering and Computer Sciences (EECS)

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Massachusetts Institute of Technology	B.S	06/04	Electrical Engineering
Massachusetts Institute of Technology	M. Eng.	06/05	Electrical Engineering and Computer Science
Massachusetts Institute of Technology	Ph.D.	08/10	Electrical Engineering and Computer Science
Princeton University	Postdoctoral	07/10-08/12	Physics and Electrical Engineering

A. Personal Statement

My research is in computational microscopy, in which optical microscopes and post-processing algorithms are designed simultaneously to create imaging systems with new capabilities. For example, we use computational illumination to image phase maps with the resolution of a ~60x objective across a field of view typical of a 2x objective, or capture large-volume 3D images with fast acquisition. We do this via simple hardware changes to commercial microscopes that are easily adoptable by other groups, combined with advanced image processing algorithms, which we make open-source, in order to promote widespread dissemination of our methods. One of our main goals is to reduce the complexity of imaging methods by transferring the main burden to computation, which is cheap and easily replicated. This enables our methods to be accessible to new applications, existing imaging systems and larger-scale data capture.

B. Positions and Honors

Positions and Employment

- 2010-2012 Postdoctoral Research Associate, Department of Electrical Engineering, Princeton University
2011-2012 Lecturer, Department of Physics, Princeton University
2012-2016 Assistant Professor, Department of EECS, UC Berkeley
Senior Fellow, Berkeley Institute of Data Science
2016- Ted Van Duzer Endowed Associate Professor, Department of EECS
Affiliate, Graduate Group in Bioengineering, Applied Science & Technology

Other Experience and Professional Memberships

- 2009- Fellow, Optical Society of America (OSA), Member SPIE, IEEE, APS
2013-2016 Member of the Board of Meetings, OSA (ad hoc Technical Group Development Chair)
2010- Conference organizing: conference Chair for 19 conferences and Program Committee for 49 conferences, including: International Conference on Computational Photography, OSA Computational Optical Sensing and Imaging, Zeiss Symposium on Optics in the Digital World, OSA Imaging Systems, ECI Photonics in Biology, Medicine and Surgery, Signal Recovery and Synthesis, SPIE Quantitative Phase Imaging, Adaptive optics and wavefront control for biology, Optics in Information Processing, SPIE 3D and Multidimensional Microscopy, SPIE Big Data Instrumentation and Management, SPIE Computational Imaging
2010- Research talks: 132 invited talks at universities, companies and conferences, including: MIT, Stanford University, UCSF, UCLA, Google, Intel, Rice, EPFL, Shanghai Institute of Optics and Fine Mechanics, SLAC, LBNL, Rice University, Harvard, Duke, Princeton, University of Arizona, University of Rochester, Carl Zeiss Inc., KLA-Tencor Inc., Qualcomm, Children's Hospital Oakland, Joint Mathematics Meeting, SIAM, CVPR, COSI, ICCP, Strata+Hadoop World

2009-	<u>Reviewer:</u> Nature, Nature Photonics, Nature Communications, ACM SIGGRAPH, Optica, Proceedings of the National Academy of Sciences, IEEE Transactions on Computational Imaging, Biomedical Optics Express, Optics Letters, Optics Express, Journal of Biomedical Optics, Applied Optics, Optics Communications, Advances in Optics and Photonics, Journal of the Optical Society of America A, Journal of Modern Optics, Proceedings of the Royal Society A, EDAS Sampling Theory and Applications, International Journal of Hydrogen Energy, Journal of Pathology Informatics, IEEE Transactions on Image Processing, International Journal of Computer Vision, ACM Transactions on Graphics
2009-	<u>Education and outreach:</u> Faculty advisor for Photobears student chapter of Optical Society (OSA), optics outreach activities for the Bay Area Science Festival and local area K-12 schools, maintain website with examples of fun optics demos and activities (20,000+ views/year)

Honors

2021	OSA Adolph Lomb Medal
2020	ASML Scientific Leadership Award, Best Student Paper (S. Sherwin), SPIE Advanced Lithography conference
2019	American Institute for Medical and Biological Engineering (AIMBE) Fellow
2019	Zeiss Best Student Poster Award (S. Sherwin), SPIE Photomask Technology conference
2019	Photronics Best Student Oral Presentation Finalist (S. Sherwin), EUV Lithography conference
2019	Outstanding Paper Award (R. Eckert), OSA Computational Optical Sensing and Imaging
2019	Best Paper Award, International Conference on Computational Photography
2018	Best Talk Award (H. Pinkard), Computational and Genomic Biology retreat
2018	OSA Fellow
2018	SPIE Early Career Achievement Award
2018	Excellence in Research Poster Award (H. Pinkard), Midwinter conference for Immunologists
2018	Best Student Paper Award (S. Dean), Imaging Systems conference
2018	Best Poster Award 2 nd place (S. Sherwin), EUV Lithography workshop
2017	Chan-Zuckerberg Initiative Biohub Investigator Award
2017	Fumio Okano Best Paper Prize, SPIE 3D Imaging, Visualization and Display conference
2017	Photronics Best Student Paper Award (S. Sherwin), SPIE BACUS conference
2017	Best Paper Award Runner-Up (Z. Phillips), OSA Imaging Systems conference
2017	Best Poster Award (M. Chen), Sculpted Light in the Brain conference
2017	Best Demo Award, International Conference on Computational Photography
2016	Agilent Early Career Professor Award Finalist for Big Data Technologies in Imaging
2016	Ted Van Duzer Endowed Chair Professorship
2016	Carol D. Soc Distinguished Graduate Student Mentoring Award for UC Berkeley
2016	Best Presentation Award, OSA Computational Optical Sensing and Imaging conference
2016	Best Paper Award, International Conference on Computational Photography
2016	National Academy of Engineering Frontiers of Engineering Participant
2014	Packard Fellowship for Science and Engineering
2014	Moore Foundation Data-driven Discovery Investigator Award
2014	NSF CAREER award
2014	Bakar Fellowship
2015	Best Student Paper Award (R. Claus), SPIE Photomask technology conference
2015	Best Student Paper Award (A. Shanker), Advanced Lithography conference
2014	Best Paper Award, Workshop in Information Optics
2014	Best Paper Award, OSA Imaging Systems conference
2011	Optical Society Outstanding Young Professional Award
2010	Award for outstanding presentation, OSA Annual Meeting
2009	Award for best contribution, International Conference on Advanced Phase Measurement

C. Contributions to Science

1. Phase Imaging: Much of my work has developed new methods for quantitative phase imaging, which provides the ability visualize transparent biological cells and map 3D shape with nanometer-level sensitivity. In contrast to typical microscope modalities (e.g. staining, fluorescence), which are either invasive or expensive, phase imaging works with unstained samples. My work uses advanced signal processing

algorithms to achieve high accuracy with very simple experimental setups that use partially-coherent illumination. This allows phase imaging methods to be implemented in existing microscopes with no hardware changes, opening up new application areas (e.g. bioimaging, X-ray metrology, lithography inspection).

- a. E.Bostan, R. Heckel, M. Chen, M. Kellman, L. Waller, "Deep Phase Decoder: self-calibrating phase microscopy with an untrained deep neural network," *Optica* 7(6), 559-562 (2020).
 - b. L.Yeh, S. Chowdhury, L. Waller, "Speckle-structured illumination for 3D phase and fluorescence computational microscopy," *Biomedical Optics Express* 10(7), 3635-3653 (2019). ***Top download**
 - c. Z. Jingshan, L. Tian, P. Varma, L. Waller, "Nonlinear optimization algorithm for partially coherent phase retrieval and source recovery," *IEEE Transactions on Computational Imaging*, PP(99), 1 (2016). ***Most Popular Paper**
 - d. L. Waller, L. Tian, G. Barbastathis, "Transport of Intensity phase-amplitude imaging with higher order intensity derivatives," *Optics Express* 18(12), 12552-12561 (2010).
2. LED Array Microscope: We have developed new computational illumination schemes and reconstruction algorithms based on the LED array microscope, in which the light source of a commercial microscope is replaced with an LED array. Each LED illuminates the sample from a different angle; this simple hardware change enables real-time phase contrast, darkfield imaging, 3D imaging, super-resolution Gigapixel microscopy and digital aberration correction. The original hardware design came from Caltech; we have built on their work in several ways: a) extending it from 2D to 3D, b) improving speed by several orders of magnitude, allowing near real-time imaging of *in vitro* samples, c) improving algorithm robustness and convergence with algorithmic self-calibration, d) enabling real-time multi-contrast on a mobile phone based microscope.
- a. L. Tian, L. Waller, "3D intensity and phase imaging from light field measurements in an LED array microscope," *Optica* (2015). ***Top cited paper 2018 and 2019**
 - b. L. Tian, X. Li, K. Ramchandran, L. Waller, "Multiplexed coded illumination for Fourier Ptychography with an LED array microscope," *Biomedical Optics Express* 5(7), 2376-2389 (2014). ***Top cited**
 - c. L. Tian, Z. Liu, M. Chen, Z. Jingshan, L. Waller, "Computational illumination for high-speed *in vitro* Fourier ptychographic microscopy," *Optica* 2(10), 904-911 (2015).
 - d. Z. Phillips, M. D'Ambrosio, L. Tian, J. Rulison, H. Patel, N. Sadras, A. Gande, N. Switz, D. Fletcher, L. Waller, "Multi-contrast imaging and digital refocusing on a mobile microscope with a domed LED array," *PLOS ONE* (2015). ***top 25% most cited**
3. Multiple-scattering Computational Microscopy: The problem of multiple scattering in imaging is generally considered intractable, due to its nonlinear nature and the massive number of degrees-of-freedom for how light propagates through an object. We are working on new unified computational and experimental approaches that will enable 3D microscopy through multiple-scattering samples, both in the optical and transmission electron microscopy (TEM) regimes.
- a. M. Chen, D. Ren, H. Liu, S. Chowdhury, L. Waller, "Multi-Layer Born multiple-scattering model for 3D phase microscopy" *Optica* 7(5), 394-403 (2020).
 - b. D. Ren, C. Ophus, M. Chen, L. Waller, "A multiple scattering algorithm for three-dimensional phase contrast atomic electron tomography," *Ultramicroscopy* 208, 112860 (2020).
 - c. S. Chowdhury, M. Chen, R. Eckert, D. Ren, F. Wu, N. Repina, L. Waller, "High-resolution 3D refractive index microscopy of multiple-scattering samples from intensity images," *Optica* 6, 1211-1219 (2019).
 - d. H. Liu, E. Jonas, L. Tian, Z. Jingshan, B. Recht, L. Waller, "3D imaging in volumetric scattering media using phase-space measurements," *Optics Express* 23(11), 14461-14471 (2015).
4. Neural Activity Tracking: We invented DiffuserCam – a lensless computational imager made solely of a random scattering element (a diffuser) placed on top of a standard image sensor. Using this simple compact device, along with advanced computational algorithms, we reconstruct 3D images with hundreds of millions of voxels, or high-speed videos, all from a single captured image. We are pursuing applications in recording electrical activity of mouse brain neurons *in vivo*. Coupled with optogenetic-like tools that cause neurons to light up when they fire, these ideas can enable dynamically tracking action potentials, with 3D cellular resolution and millisecond precision.
- a. N. Pegard, H. Liu, N. Antipa, M. Gerlock, H. Adesnik, L. Waller, "Compressive light-field microscopy for 3D neural activity recording," *Optica* 3(5), 517-524 (2016). ***Optics & Photonics highlight**

- b. N. Pegard, A. Mardinly, I. Oldenburg, S. Sridharan, L. Waller, H. Adesnik, "Three-dimensional scanless holographic optogenetics with temporal focusing (3D-SHOT)," *Nature Communications* 8:1228 (2017).
 - c. A. Mardinly, I. Oldenburg, N. Pegard, S. Sridharan, E. Lyall, K. Chesnov, S. Brohawn, L. Waller, H. Adesnik, "Precise multimodal optical control of neural ensemble activity," *Nature Neuroscience* (2018).
 - d. N. Antipa, G. Kuo, R. Heckel, E. Bostan, B. Mildenhall, R. Ng, L. Waller, "DiffuserCam: lensless single-exposure 3D imaging," *Optica* 5(1), 1-9 (2017). ***top download**
5. **Machine Learning for Optical System Design:** We are applying machine learning tools for reconstruction of high-dimensional images; for example, in the multiple scattering problem it is not possible to exhaustively search the billions of possible scattering paths, but machine learning tools can selectively explore huge solution spaces and use training or prior knowledge to converge on the correct one. Beyond this, we are working on using neural networks for end-to-end design of optical systems. Our physics-based learned design technique allows one to solve for both the optimal system hardware design and the optimal image reconstruction technique, by incorporating known physics into machine learning pipelines to extract the efficiencies of both.
- e. K. Monakhova, J. Yurtsever, G. Kuo, N. Antipa, K. Yanny, L. Waller, "Learned reconstructions for practical mask-based lensless imaging," *Optics Express* 27, 28075-28090 (2019).
 - f. M. Kellman, E. Bostan, N. Repina, L. Waller, "Physics-based learned design: Optimized coded-illumination for quantitative phase imaging," *IEEE Transactions of Computational Imaging* 5(3), 344-353 (2019). ***Featured article**
 - g. E. Bostan, U. Kamilov L. Waller, "Learning-based image reconstruction via parallel proximal algorithm," *IEEE Signal Processing Letters* 25(7), 989-993 (2018).

Complete List of Published Work

<http://www.laurawaller.com/publications/>

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Rikky Muller, PhD

ERA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology (MIT)	S.B.	2004	Electrical Engineering
Massachusetts Institute of Technology (MIT)	M.Eng.	2004	Electrical Engineering
University of California, Berkeley	Ph.D.	2013	Electrical Engineering

A. Personal Statement

The focus of my research is to develop new and revolutionary microsystems that directly interface with the brain, monitor and treat neurological disorders, enable fundamental neuroscientific discovery. Today, neuroscientists and clinical researchers lack the tools they need for the research of neurological disorders. My research develops tools for advanced study of the brain and nervous system, as well as autonomous devices will monitor, diagnose, treat and in the long term even cure disease. In particular my group is focused on closed-loop neurostimulation technologies that involve concurrent sensing and stimulation. We have developed artefact-free, wireless closed-loop deep brain stimulation (*Nature BME* 2018), as well as ~mm³ ultrasonic motes for stimulation (*Nature BME* 2020) and sensing (*IEEE JSSC* 2019) in the peripheral nervous system. Our group is currently developing a class of optical devices (*Nature LSA*, 2020) for high-speed holographic photostimulation. We are also working on low-power, efficient machine learning algorithms and circuits for closing the loop (*IEEE VLSI* 2021). The devices I develop are hybrid integrated circuits/MEMS systems that are miniaturized, wireless, remotely powered and have embedded intelligence to learn the signatures of disease and treat on demand. Such tools will enable a wealth of patient data that cannot be accessed today and will open the door to significant scientific discovery and new treatment paradigms of neurological disorders.

B. Positions and Honors**University of California, Berkeley**

Department of Electrical Engineering and Computer Sciences

S. Shankar Sastry Assistant Professor in Emerging Technologies

- Co-director, Berkeley Wireless Research Center (BWRC)
- Core member, Center for Neural Engineering and Prostheses (CNEP)
- Investigator, Chan-Zuckerberg Biohub

Jan 2016 – Present

Cortera Neurotechnologies, Inc.

Consultant & Chair of the Board

Mar 2013 – July 2019

Chief Technology Officer

Nov 2014 – Jan 2016

Chief Executive Officer

Mar 2013 – Nov 2014

- Developed high performance, minimally invasive neural interfaces for clinical applications, acquired in 2019

Nia Therapeutics, Inc.

Technical consultant

July 2019 – Present

University of Melbourne	Sep 2013 – Jun 2014
Lecturer and McKenzie Postdoctoral Fellow of Electrical and Electronic Engineering	
Berkeley Wireless Research Center	Aug 2007–May 2013
Graduate Research Assistant, Ultra-low Energy Research.	
• Led collaborative project in wireless Brain-Machine Interfaces; developed wireless micro-ECoG device	
Analog Devices, Inc.	Jul 2004 – Jul 2007
Integrated Circuit Design Engineer, High-Speed Linear Products.	

MIT Research Laboratory of Electronics	Jun 2003 – Jun 2004
Graduate Research Assistant, BioMEMS Group.	

Recent Fellowships, Awards

- IEEE Solid-State Circuits Society New Frontiers Award, 2021
- McKnight Technological Innovations in Neuroscience Award, 2020
- Bakar Fellowship 2020-2021
- N2Women: Rising Stars in Computer Networking and Communications List 2020
- IEEE Solid-State Circuits Society Distinguished Lecturer, 2020-2021
- S. Shankar Sastry Professorship in Emerging Technologies – Endowed Chair 2019-2024
- NSF CAREER Award 2019-2023
- Hellman Fellowship 2019-2020
- Fung Institute Most Innovative Project Award, 2018
- Chan-Zuckerberg Biohub Investigatorship, 2017-2022
- Keysight Early Career Professor Award, 2017
- National Academy of Engineering Gilbreth Lectureship, 2017
- Melbourne School of Engineering Visiting Fellowship, 2017
- Boston Medtech's 40 Under 40, 2016
- MIT Technology Review TR35, Top Innovator Under 35, 2015
- McKenzie Postdoctoral Fellowship 2014-2017
- Melbourne Accelerator Program (MAP) Entrepreneurial Fellowship 2013

C. Contributions to Science

1. AJ Zhou, SR Santacruz, BC Johnson, GP Alexandrov, A Moin, FL Burghardt, JM Rabaey, JM Carmena, R Muller. "[A wireless and artifact-free 128-channel neuromodulation device for closed-loop stimulation and recording in non-human primates](#)" *Nature Biomedical Engineering*, 3 (1), Dec. 2018, pp. 15-26. [[Supplementary information](#)]

This paper describes the Wireless Artifact-Free Neuromodulation Device, which utilizes two of the integrated circuits described above. Existing systems for wireless recording and stimulation are limited by low channel counts, lack of algorithmic flexibility, and distortion of recorded signals from large, persistent stimulation artifacts. Here, we describe a device that enables new research applications requiring high-throughput data streaming, low-latency biosignal processing, and truly simultaneous sensing and stimulation. WAND is a miniaturized, wireless neural interface capable of recording and stimulating on 128 channels with on-board processing to fully cancel stimulation artifacts, detect neural biomarkers, and automatically adjust stimulation parameters in a closed-loop fashion. It combines our custom ASICs with an on-board FPGA for programmability of closed-loop algorithms, and a low-power bidirectional radio. We validate wireless, long-term recordings of local field potentials (LFP) and real-time cancellation of stimulation artifacts in a behaving nonhuman primate (NHP). We use WAND to demonstrate a closed-loop stimulation paradigm to disrupt movement preparatory activity during a delayed-reach task in a NHP *in vivo*. WAND utilizes a custom application specific integrated circuit (ASIC) for highly scaled closed-loop Neurostimulation (NS). A state-of-the-art NS device applies periodic zero-mean current pulses to the brain, with an amplitude and frequency of stimulation determined heuristically by a clinician. Recently, NS devices have added the ability to record neurological signals and stimulate

in response to specific biomarkers *in situ*. Concurrent sensing and stimulation remains a great challenge in enabling closed-loop operation since stimulation artifacts can saturate amplifiers, create large and long-lasting artifacts and corrupt the signal. This device enables concurrent sensing and stimulation on many channels without artefact.

2. DK Piech, BC Johnson, K Shen, MM Ghanbari, KY Li, R Neely, JM Carmena, MM Maharbiz, R Muller. "[A wireless millimetre-scale implantable neural stimulator with ultrasonically powered bidirectional communication](#)" *Nature Biomedical Engineering*, [[Supplementary information](#)], 4 (2), Feb. 2020, pp. 207-222.

This paper describes a 1.7mm³, 10mg, wireless peripheral nerve stimulator. The stimulator is powered and controlled through ultrasound from an external transducer and utilizes a single 0.42 mm³ piezocrystal for downlink communication, powering, and readout, reducing implant volume and mass. An IC with 0.06 mm² active circuit area, designed in TSMC 65nm LPCMOS process, converts harvested ultrasound to stimulation charge with a peak efficiency of 82%. A custom wireless protocol that does not require a clock or memory circuits reduces on-chip power to 4 microwatts when not stimulating. The encapsulated stimulator was cuffed to the sciatic nerve of an anesthetized rodent and demonstrated full-scale nerve activation *in vivo*. We achieve a highly efficient and temporally precise wireless peripheral nerve stimulator that is the smallest and lightest to our knowledge.

3. NT Ersimo, C Yalcin, N Antipa, N Pégard, L Waller, D Lopez, and R Muller, "[A micromirror array with annular partitioning for high-speed random-access axial focusing](#)," *Light: Science & Applications*, vol. 9, no. 1, 2020.

Optical systems expand their 2D frame basis to tackle 3D addressing by dynamically tuning focal distances, bringing different depths into focus for volumetric sweeping (ex: biological scanning microscopy) or for human/machine vision (ex: AR/VR). Yet state-of-the-art dynamic focusing tools sacrifice either speed, random-access, or ease of operation. To address these bottlenecks, this work develops a compact, varifocal array based on fast-switching micromirrors that can accommodate a wide range of wavelengths for imaging, projection and biology. The array is driven with low CMOS compatible voltages across only 32 channels. By designing fast-response microelectromechanical mirrors in unit structures for high sensitivity, tiling these structures into an array of nearly 24,000 pixels, and partitioning these pixels into 32 annular ring groups, this work achieves both robust, wide-range focus tuning and a driving system that is lightweight and compact enough for on-board integration. The array can switch at speeds higher than 15 kHz and accommodate wavelengths of up to 1100 nm while only requiring a maximum drive of 30 V. Altogether, this device achieves a speed that is 2 orders of magnitude greater than conventional dwelling-capable varifocal systems with a channel count and voltage range that is 1-2 orders of magnitude smaller than existing deformable mirror arrays.

4. R Muller, H-P Le, W Li, P Ledochowitsch, S Gambini, T Borjninien, A Koralek, JM Carmena, MM Maharbiz, E Alon, JM Rabaey. "[A Minimally Invasive 64-channel Wireless uECoG Implant](#)" *IEEE Journal of Solid-State Circuits (JSSC)*, Special Issue of the ISSCC, 50 (1), Jan. 2015, pp.1-16.

This paper demonstrates a miniaturized, flexible, 64-channel, wireless electrocorticography (ECoG) microsystem. We demonstrate, for the first time, a 64-channel recording platform that can be chronically implanted and powered well within established IEEE and FCC limits, on a flexible substrate and occupying a compact, mm-scale form factor. For these reasons, this approach has excellent prospect to become the technology of choice for clinically relevant neural recording for the study and continuous monitoring of neurological conditions. This paper discusses the specific circuit techniques used and implemented to achieve extreme miniaturization and power efficiency in the wireless ECoG implant system. Particularly, a new wireless protocol using a single link for power and data is discussed, as well as techniques in the sensor interface circuits that are the most power and area efficient achieved to our knowledge.

5. R Muller, S Gambini, JM Rabaey, "[A 0.013mm², 5μW, DC Coupled Neural Signal Acquisition IC with 0.5V Supply](#)" *IEEE Journal of Solid-State Circuits (JSSC)*, Special Issue of the ISSCC, Vol. 47, Issue 1, Jan. 2012, pp. 232-243.

Neuroscience research is limited by the available tools for sensing neurological activity and their limited scale. Scaling implantable neural interfaces to hundreds or thousands of recording sites is fundamentally limited by the power and size of the implant. The largest and most power-hungry element in these systems is the neural signal acquisition circuitry, which historically has not scaled well. I invented a new architecture for these acquisition circuits that reduced their size by over an order of magnitude and allowed them to be powered off of a lower voltage making the integration of a large number of channels practical in a very small form factor. This work remains the state of the art in neural amplifier/front-end designs and is one of the most highly cited papers in the area of neurological sensing hardware.

D. Research Support

NSF	02/15/2019 – 01/31/2024
<i>CAREER: Intelligent, Closed-Loop Neural Interfaces</i>	\$500,000
Chan Zuckerberg Foundation	04/01/2017 – 03/31/2022
Chan Zuckerberg Biohub Investigator Fellow	\$750,000
Weill Neurohub	04/01/2020 – 03/31/2022
Optimizing Electrical Stimulation to Target Neural Population Dynamics	\$230,000
Bakar Fellows Program	09/15/2020 – 09/14/2022
Ear EEG: Hearables that Read Your Mind	\$150,000
McKnight Foundation	08/01/2020 - 07/31/2022
High-speed Holography for Optogenetic Control of Thousands of Neurons	\$200,000
Boulder Nonlinear Systems, NIH	08/01/2020 - 07/31/2022
A MEMS-based High-Throughput Photostimulation Device with Commercial Backplane Integration	
\$128,000	

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Michael Lin

ERA COMMONS USER NAME (credential, e.g., agency login): LIN.MICHAEL

POSITION TITLE: Associate Professor of Neurobiology and Bioengineering

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Harvard College, Cambridge, Massachusetts	A.B. <i>summa cum laude</i>	06/1994	Biochemistry
Harvard Medical School, Boston, Massachusetts	Ph.D.	06/2002	Biological and Biomedical Sciences
UCLA School of Medicine, Los Angeles, California	M.D.	06/2004	Medicine
UCSD School of Medicine, La Jolla, California	postdoctoral	05/2009	Pharmacology and Neuroscience

A. Personal Statement

My research interests are at the interface of protein chemistry, protein engineering, and genetics. I received training with Dr. Michael Greenberg on biochemical regulation of neuronal differentiation and synaptic function, and with Dr. Roger Tsien on fluorescent protein chemistry and engineering. My laboratory has pioneered the use of structure-based protein engineering to develop fluorescent and luminescent reporters for deep-tissue imaging in animals, and, most relevantly to this project, has designed the ASAP family of fast fluorescent genetically encoded voltage indicators which have been widely used in flies, fish, and mammals for 1-photon and 2-photon imaging of electrical activity in the brain.

The ASAP-family voltage indicators produce the largest photonic responses of genetically encoded voltage indicators characterized to date while tracking action potentials with sub-millisecond time resolution. We have used ASAPs with high-speed 2-photon scanning methods to record electrical activity in fly and mice brains, in work reported in *Nature Neuroscience* in 2014, *Cell* in 2016, and *Cell* in 2019. We have further pioneered GEVI engineering methods by introducing direct PCR transfection and electroporation for library expression and screening.

We are committed to educating neuroscientist on voltage imaging and enabling the study of neuronal computation in awake animals. We have disseminated our experience in fluorescent protein chemistry and reporter development in reviews of genetically encoded neuronal activity indicators. We are providing pre-publication ASAP-family indicators and experimental advice to numerous neuroscience labs around the world.

Four research outcomes that specifically highlight my qualifications for this project are:

1. Wu J, Liang Y, Chen S, Hsu C, Chavarha M, Evans SW, Shi D, **Lin MZ**, Tsia KK, Ji N. **2020**. Kilohertz *in vivo* imaging of neural activity. ***Nature Methods*** 17:287. <http://doi.org/10.1038/s41592-020-0762-7>.
2. Villette V*, Chavarha C*, Dimov IK, Bradley J, Pradhan L, Mathieu B, Evans SW, Chamberland S, Shi D, Yang R, Kim BB, Ayon A, Jalil A, St-Pierre F, Schnitzer MJ, Bi G, Toth K, Ding J, Dieudonné S, **Lin MZ**. **2019**. Ultrafast two-photon imaging of a high-gain voltage indicator in awake behaving mice. ***Cell*** 179:1590. <http://doi.org/10.1016/j.cell.2019.11.004>. PMID 31835034. *equal contribution.
3. Yang HH*, St-Pierre F*, Sun X, Ding X, **Lin MZ**, Clandinin TR. **2016**. Subcellular imaging of voltage and calcium signals reveals neural processing *in vivo*. ***Cell*** 166:1-16. PMID: 27264607. PMCID: PMC5606228. DOI: 10.1016/j.cell.2016.05.031. *co-first authors.

4. St-Pierre F, Marshall JD, Yang Y, Gong Y, Schnitzer MJ, **Lin MZ**. 2014. High-fidelity optical reporting of neuronal electrical activity with an ultrafast fluorescent voltage sensor. *Nature Neuroscience* 17:884-889. PMID: 24755780. PMCID: PMC4494739. DOI: 10.1038/nn.3709.

B. Positions and Honors

Positions

- 1992-1994: Undergraduate research assistant, Laboratory of Dr. Fotis Kafatos, Dept. of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts
- 1994-1996: Medical student, Laboratory of S. Larry Zipursky, Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, California
- 1996-2002: Graduate research associate, Laboratory of Dr. Michael E. dGreenberg, Division of Neuroscience, Children's Hospital, Boston, Massachusetts
- 2002-2004: Medical student, UCLA School of Medicine, Los Angeles, California
- 2004-2009: Postdoctoral research fellow, Laboratory of Dr. Roger Y. Tsien, Dept. of Pharmacology and Howard Hughes Medical Institute, University of California at San Diego, La Jolla, California
- 2009-2017: Assistant Professor of Pediatrics and Bioengineering and, by courtesy, Chemical and Systems Biology, Stanford University School of Medicine, Stanford, California
- 2017-current: Associate Professor of Neurobiology and Bioengineering and, by courtesy, Chemical and Systems Biology, Stanford University School of Medicine, Stanford, California

Honors

- Roger Tsien Award for Excellence in Chemical Biology, World Molecular Imaging Society, 2019
- NIH Pioneer Award, 2013
- Rising Star Award, Biomedical Engineering Society, Cellular and Molecular Bioengineering Group, 2013
- Damon Runyon-Rachleff Innovation Award, November 2012
- Rita Allen Scholar Award, 2011
- Young Investigator Award, Alliance for Cancer Gene Therapy, 2010
- Burroughs Wellcome Career Award for Medical Scientists, 2007
- University of Illinois at Urbana Champagne Young Excellence in Engineering Seminar, 2006
- Jane Coffin Childs Memorial Fund for Medical Research Fellowship, 2005
- United States Department of Defense Graduate Fellowship, 1996
- NIH Medical Scientist Training Program Scholarship, 1994
- Harvard University Phi Beta Kappa, 1994

Other Experience, Professional Memberships and Certifications

- 2011-current: Member, American Society for Biochemistry and Molecular Biology
- 2008-current: Member, Biophysical Society
- 2008-current: Member, American Society for Cell Biology
- 2006-current: Member, Society for Neuroscience
- USMLE Step I and II certified

C. Contribution to Science

1. A major contribution of my research has been the development of indicators of membrane voltage based on circularly permuted GFP. These ASAP-family indicators uniquely combine fast activation kinetics, large response amplitudes, and compatibility with two-photon imaging, and have been used to detect voltage kinetics in neurons in living animals. They are currently the only genetically encoded voltage indicators that can reveal electrical activity under two-photon microscopy, and thus are well suited for recording voltage in individual neurons deep in the brain.

Villette V*, Chavarha C*, Dimov IK, Bradley J, Pradhan L, Mathieu B, Evans SW, Chamberland S, Shi D, Yang R, Kim BB, Ayon A, Jalil A, St-Pierre F, Schnitzer MJ, Bi G, Toth K, Ding J, Dieudonné S, **Lin MZ**. 2019.

Ultrafast two-photon imaging of a high-gain voltage indicator in awake behaving mice. **Cell** 179:1590. <http://doi.org/10.1016/j.cell.2019.11.004>. PMID 31835034. *equal contribution.

Lin MZ*, Schnitzer MJ*. **2016**. Genetically encoded indicators of neuronal activity. **Nature Neuroscience** 19:1142-1153. PMID: 27571193. PMCID: PMC5557009. DOI: 10.1038/nn.4359. *corresponding authors.

Yang HH*, St-Pierre F*, Sun X, Ding X, **Lin MZ**, Clandinin TR. **2016**. Subcellular imaging of voltage and calcium signals reveals neural processing in vivo. **Cell** 166:1-16. PMID: 27264607. PMCID: PMC5606228. DOI: 10.1016/j.cell.2016.05.031. *co-first authors.

St-Pierre F, Marshall JD, Yang Y, Gong Y, Schnitzer MJ, **Lin MZ**. **2014**. High-fidelity optical reporting of neuronal electrical activity with an ultrafast fluorescent voltage sensor. **Nature Neuroscience** 17:884-889. PMID: 24755780. PMCID: PMC4494739. DOI: 10.1038/nn.3709.

2. Another major contribution of my research has been the engineering of new fluorescent proteins to enable new modes of imaging in cells. For example, my lab created monomeric green and red fluorescent proteins (mClover3 and mRuby3) that were the brightest in their wavelength classes, and used these to improve the performance of FRET-based reporters of biochemical activities. We also engineered cyan-exitable orange-red fluorescent proteins (CyOFPs) for simultaneous two-photon microscopy with GFP. Finally, we created a bioluminescent reporter protein composed of the highly catalytic blue luciferase NanoLuc fused to two copies of CyOFP1. The resulting protein, Antares, uses resonance energy transfer from NanoLuc to CyOFP1 to produce bright red emission that for the first time achieved brightness superior to firefly luciferase in mice. We subsequently created a calcium-dependent form of Antares, named CaMBI, to report calcium transients from deep tissues in mice in a completely non-invasive manner.

Oh Y, Park Y, Cho JH, Wu H, Paultk NK, Liu LX, Kim N, Kay MA, Wu JC, **Lin MZ**. **2019**. An orange calcium-modulated bioluminescent indicator for non-invasive activity imaging. **Nature Chemical Biology** 15:433-436. PMID: 30936501. PMCID: PMC6563924. DOI: 10.1038/s41589-019-0256-z.

Laviv T*, Kim BB*, Chu J, **Lin MZ**, Yasuda R. **2016**. Simultaneous dual-color fluorescence lifetime imaging with novel red-shifted fluorophores. **Nature Methods** 13:989-992. PMID: 27798609. PMCID: PMC5322478. DOI: 10.1038/nmeth.4046. *co-first authors.

Chu J, Oh YH, Sens A, Ataei N, Dana H, Macklin J, Laviv T, Welf ES, Dean KM, Zhang F, Kim BB, Tang CT, Hu M, Baird MA, Davidson MW, Fioka F, Kay M, Fioka R, Yasuda R, Kim DS, Ng H-L, **Lin MZ**. **2016**. A bright cyan-exitable orange fluorescent protein enables dual-emission microscopy and highly sensitive bioluminescence imaging in vivo. **Nature Biotechnology** 34:760. PMID: 27240196. PMCID: PMC4942401. DOI: 10.1038/nbt.3550.

Lam AJ, St-Pierre F, Gong Y, Marshall JD, Cranfill PJ, Baird MA, McKeown MR, Wiedenmann J, Davidson MW, Schnitzer MJ, Tsien RY, **Lin MZ**. **2012**. Improving FRET dynamic range with bright green and red fluorescent proteins. **Nature Methods** 9:1005-1012. PMID: 22961245; PMCID: PMC3461113. DOI: 10.1038/nmeth.2171.

3. Another major scientific contribution of my work has been the development of the first generalizable method for making light-regulatable proteins, which I believe will allow optical regulation of biology (optobiology) to become a commonplace experimental approach. As part of this work, we discovered that the oligomerization state of an engineered photoswitching fluorescence protein can be controlled by light, in the first example of a light-regulatable protein-protein interaction outside of natural light-regulated signal transduction pathways. We then used this behavior to create a generalizable design for light-controllable proteins in which a protein is caged by fusion to fluorescent protein domains and uncaged by light.

Zhou XX, Fan LZ, Li P, Shen K, **Lin MZ**. **2017**. Optical control of cell signaling by single-chain photoswitchable kinases. **Science** 355:836-842. PMID: 28232577. PMCID: PMC5589340. DOI: 10.1126/science.aah3604.

Zhou XX, Zou X, **Lin MZ**. **2017**. A single-chain photoswitchable Cas9 architecture for inducible gene editing and transcription. **ACS Chem Biol** 13:443-448. PMID: 28938067. PMCID: PMC5820652. DOI: 10.1021/acschembio.7b00603.

Zhou XX, Pan M, **Lin MZ**. **2015**. Investigating neuronal development and function with engineered light-controllable proteins. **Frontiers in Molecular Neuroscience**. PMID: 26257603. PMCID: PMC4543534. DOI: 10.3389/fnmol.2015.00037.

Zhou XX, Chung HK, Lam AJ, **Lin MZ**. **2012**. Optical control of protein activity by fluorescent protein domains. **Science** 338:810-814. PMID: 23139335. PMCID: PMC3702057. DOI: 10.1126/science.1226854.

4. My lab also explores protein design and evolution to create monomeric far-red fluorescent proteins. We demonstrated that a bright far-red fluorescent protein was superior to other fluorescent proteins for non-invasive longitudinal imaging of stem cell differentiation in living mice, and that further red-shifting allow orthogonal imaging with orange fluorescent proteins.

Bajar BT*, Lam AJ*, Badiée R, Oh YH, Chu J, Zhou XX, Kim N, Kim BB, Chung M, Yabonovitch AL, Cruz BF, Kulalert K, Tao JJ, Meyer T, Su XD, **Lin MZ**. 2016. Fluorescent indicators for simultaneous reporting of all four cell cycle phases. *Nature Methods* 13:993-996. PMID: 27798610. PMCID: PMC5548384. DOI: 10.1038/nmeth.4045. *co-first authors.

Chu J, Haynes RD, Corbel SY, Li P, González-González E, Burg JS, Ataie NJ, Lam AJ, Cranfill PJ, Baird MA, Davidson MW, Ng HL, Garcia KC, Contag CH, Shen K, Blau HM, **Lin MZ**. 2014. Non-invasive intravital imaging of cellular differentiation with a bright red-exitable fluorescent protein. *Nat Methods* 11:572-578. PMID: 24633408. PMCID: PMC4008650. DOI: 10.1038/nmeth.2888.

Lin MZ, McKeown MR, Aguilera T, Shaner NC, Campbell RE, Adams SR, Tsien RY. 2009. Autofluorescent proteins with excitation in the optical window for intravital imaging in mammals. *Chemical Biology* 16:1169-1179. PMID: 19942140. PMCID: PMC2814181. DOI: 10.1016/j.chembiol.2009.10.009.

Shu X, Royant A, **Lin MZ**, Aguilera T, Levram-Ellisman V, Steinbach PA, Tsien RY. 2009. Mammalian expression of infrared fluorescent proteins engineered from a bacterial phytochrome. *Science* 324:804-807. Erratum in: *Science* 326:1482. PMID: 19423828. PMCID: PMC2763207. DOI: 10.1126/science.1168683.

5. Another major contribution has been the development of reporters of newly synthesized proteins. We developed TimeSTAMP tags that selectively label new copies of a protein of interest, enabling studies of where new copies of specific proteins are distributed. In TimeSTAMP, the hepatitis C virus (HCV) protease and an epitope tag or fluorescent protein is fused to a protein of interest via the specific protease substrate site. By default, cleavage of the substrate site removes the tag or destroys the fluorescent protein. After addition of a HCV protease inhibitor, however, newly synthesized copies of the protein of interest remain labelled with the tag or fluorescent protein, and thus can be visualized by immunofluorescence or direct fluorescence. We discovered the synaptic structural protein PSD95 is synthesized in response to activity and selectively localized at activated synapses, which may represent the first case of a protein to demonstrably meet the definition of a synaptic learning tag. I conceived and developed the TimeSTAMP epitope tag while a postdoctoral fellow with Dr. Roger Tsien, then generalized it to live fluorescent protein readouts and used it to visualize activity-dependent new PSD95 expression as an assistant professor. Most recently we used TimeSTAMP to discover perturbations in new PSD95 expression in dendrites in FMRP-knockout neurons, and demonstrate rescue of this molecular phenotype using inhibitors of the mTORC1-S6K1 pathway.

Yang Y, Geng Y, Jiang D, Ning L, Kim HJ, Jeon NL, Lau A, Chen L, **Lin MZ**. 2019. Kinase pathway inhibition restores PSD95 induction in neurons lacking fragile X mental retardation protein. *Proc Natl Acad Sci* 24:12007-12012. PMID: 31118285. DOI: 10.1073/pnas.1812056116.

Butko MT, Yang J, Geng Y, Kim HJ, Jeon NL, Shu X, Mackey MR, Ellisman MH, Tsien RY, **Lin MZ**. 2012. Fluorescent and photo-oxidizing TimeSTAMP tags track protein fates in light and electron microscopy. *Nature Neuroscience* 15:1742-51. PMID: 23103964. PMCID: PMC3509268. DOI: 10.1038/nn.3246.

De Jaco A, **Lin MZ**, Dubi N, Comoletti D, Miller M, Camp S, Ellisman M, Bukto MT, Tsien RY, Taylor P. 2010. Neuroligin trafficking deficiencies arising from mutations in the alpha/beta-hydrolase fold family. *J Biological Chemistry* 10:28674-82. PMID: 20615874. PMCID: PMC2937894. DOI: 10.1074/jbc.M110.139519.

Lin MZ, Glenn JS, Tsien RY. 2008. A drug-controllable tag for specific labeling of newly synthesized proteins in cells and whole animals. *Proc Natl Acad Sci* 105:7744-9. PMID: 18511556. PMCID: PMC2402386. DOI: 10.1073/pnas.0803060105. (Cover article.)

A complete list of publications is at <https://www.ncbi.nlm.nih.gov/myncbi/michael.lin.1/bibliography/public/>

D. Selected Research Support

Ongoing Research Support

NIH/NINDS 1U01NS103464	08/01/2017 – 07/30/2020 (+1-year NCE)
“Bringing laser focus to voltage imaging: Enhanced indicators and advanced scanning methods for two-photon recording of dense networks <i>in vivo</i> ”	
Major goals: (1) develop red-emitting, subcellularly localized, and ratiometric voltage indicators; (2) develop random-access multi-photon microscopy with motion correction and enhanced throughput; (3) record voltage from hundreds of neurons <i>in vivo</i> with millisecond precision.	
Responsibility: PI	
NIH/NIMH 1RF1MH114105	08/08/2017 – 08/07/2020 (+1-year NCE)
“Revealing circuit control of neuronal excitation with next-generation voltage indicators”	
Major goals: (1) generate brighter and more responsive variants of the voltage indicators ASAP2s and Ace-mNeonGreen; (2) validate voltage indicators for reporting contributions of specific inputs to subthreshold and action potential responses in fly neurons <i>in vivo</i> ; (3) test indicator performance under two-photon excitation in striatal spiny projection neurons in acute brain slices and in living mice.	
Responsibility: PI	
NIH/NIMH 1R01MH114227 (PI: Jin Hyung Lee)	08/09/2017 – 08/08/2021
“Defining cell type specific contributions to fMRI signals”	
Major goals: (1) determine D1 and D2 medium spiny neuron (MSN)-driven fMRI and electrophysiology signals in the brain; (2) define specific cells activated by D1 and D2 MSN; (3) computationally model cell type-specific contributions to D1 and D2 MSN-driven activity.	
Responsibility: Co-investigator	

Completed Research Support

NIH 5DP1GM111003-02 (PI: Lin)	10/1/2013 – 07/31/2018
“Optogenetics For All: A General Method for Optical Control of Protein Activity”	
Major goals: (1) to characterize the fluorescent light-inducible protein architecture; (2) to extend the phenomenon of light-induced dissociation of fluorescent protein domains to target proteins with various topologies; (3) to determine whether fluorescent light-induced proteins can be used to control neuronal development in living animals.	
Responsibility: PI	
NIH/NINDS 1U01NS090600-01	9/1/2014 – 8/31/2017
“Protein Voltage Sensors: Kilohertz Imaging of Neural dynamics in behaving animals”	
Major goal: Engineering novel voltage-imaging capabilities for use in fluorescence imaging of neural activity.	
Responsibility: Co-PI	

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California

Start Date*: 02-01-2022

End Date*: 01-31-2023

Budget Period: 1

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Hillel		Adesnik		PD/PI				1.0	14,967.00	2,155.00	17,122.00
2.	Na		Ji		Co-Investigator				0.1	1,661.00	240.00	1,901.00
3.	Stephen		Brohawn		Co-Investigator				0.1	1,286.00	185.00	1,471.00
4.	Laura		Waller		Co-Investigator				0.1	1,661.00	240.00	1,901.00
5.	Rikki		Muller		Co-Investigator				1.0	14,222.00	2,048.00	16,270.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	38,665.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
5	Post Doctoral Associates	36.0			178,012.00	25,633.00	203,645.00
2	Graduate Students		8.9	4.5	91,592.00	59,469.00	151,061.00
	Undergraduate Students						
	Secretarial/Clerical						
2	Staff Research Associate	9.0			40,335.00	17,668.00	58,003.00
9	Total Number Other Personnel					Total Other Personnel	412,709.00
						Total Salary, Wages and Fringe Benefits (A+B)	451,374.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 02-01-2022

End Date*: 01-31-2023

Budget Period: 1

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

	Funds Requested (\$)*
1 . Table optics and optomechanics	45,000.00
2 . Meadowlark Optics HD SLM	24,000.00
3 . Hnu Nuvu 128 EMCCD camera	40,000.00
4 . Coherent Monaco 60W/Opera-F OPA system	275,000.00

Total funds requested for all equipment listed in the attached file

Total Equipment 384,000.00

Additional Equipment: File Name:

D. Travel

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	Funds Requested (\$)*
2. Foreign Travel Costs	14,000.00

Total Travel Cost 14,000.00

E. Participant/Trainee Support Costs

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees

Total Participant Trainee Support Costs

0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 02-01-2022

End Date*: 01-31-2023

Budget Period: 1

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		96,470.00
2. Publication Costs		2,000.00
3. Consultant Services		
4. ADP/Computer Services		6,356.00
5. Subawards/Consortium/Contractual Costs		208,966.00
6. Equipment or Facility Rental/User Fees		20,000.00
7. Alterations and Renovations		
	Total Other Direct Costs	333,792.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	1,183,166.00

H. Indirect Costs		Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
Indirect Cost Type				
1 . Modified Total Direct Costs		60.5	558,113.00	337,658.00
			Total Indirect Costs	337,658.00
Cognizant Federal Agency	DHHS Cost Allocation Services, Arif M. Karim, 415-437-7820			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	1,520,824.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		1,520,824.00

L. Budget Justification*	File Name: 29639_BudgetJustification_FINAL.pdf (Only attach one file.)
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California

Start Date*: 02-01-2023

End Date*: 01-31-2024

Budget Period: 2

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Hillel		Adesnik		PD/PI				1.0	14,967.00	2,155.00	17,122.00
2.	Na		Ji		Co-Investigator				0.1	1,661.00	240.00	1,901.00
3.	Stephen		Brohawn		Co-Investigator				0.1	1,286.00	185.00	1,471.00
4.	Laura		Waller		Co-Investigator				0.1	1,661.00	240.00	1,901.00
5.	Rikki		Muller		Co-Investigator				1.0	14,791.00	2,129.00	16,920.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	39,315.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
5	Post Doctoral Associates	36.0			184,282.00	26,535.00	210,817.00
2	Graduate Students		8.9	4.5	93,581.00	61,006.00	154,587.00
	Undergraduate Students						
	Secretarial/Clerical						
2	Staff Research Associate	9.0			40,335.00	17,668.00	58,003.00
9	Total Number Other Personnel					Total Other Personnel	423,407.00
						Total Salary, Wages and Fringe Benefits (A+B)	462,722.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: Project Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 02-01-2023

End Date*: 01-31-2024

Budget Period: 2

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

	Funds Requested (\$)*
1 . Memscap	67,511.00
2 . T-Micro	12,450.00

Total funds requested for all equipment listed in the attached file

Total Equipment 79,961.00

Additional Equipment: File Name:

D. Travel

	Funds Requested (\$)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	14,000.00
2. Foreign Travel Costs	
Total Travel Cost	<u>14,000.00</u>

E. Participant/Trainee Support Costs

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees Total Participant Trainee Support Costs 0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 02-01-2023

End Date*: 01-31-2024

Budget Period: 2

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		90,651.00
2. Publication Costs		4,000.00
3. Consultant Services		
4. ADP/Computer Services		6,356.00
5. Subawards/Consortium/Contractual Costs		212,887.00
6. Equipment or Facility Rental/User Fees		20,000.00
7. Alterations and Renovations		
	Total Other Direct Costs	333,894.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	890,577.00

H. Indirect Costs		Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
Indirect Cost Type				
1 . Modified Total Direct Costs		60.5	539,156.00	326,189.00
			Total Indirect Costs	326,189.00
Cognizant Federal Agency	DHHS Cost Allocation Services, Arif M. Karim, 415-437-7820			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	1,216,766.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		1,216,766.00

L. Budget Justification*	File Name: 29639_BudgetJustification_FINAL.pdf (Only attach one file.)
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California

Start Date*: 02-01-2024

End Date*: 01-31-2025

Budget Period: 3

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Hillel		Adesnik		PD/PI				1.0	14,967.00	2,155.00	17,122.00
2.	Na		Ji		Co-Investigator				0.1	1,661.00	240.00	1,901.00
3.	Stephen		Brohawn		Co-Investigator				0.1	1,286.00	185.00	1,471.00
4.	Laura		Waller		Co-Investigator				0.1	1,661.00	240.00	1,901.00
5.	Rikki		Muller		Co-Investigator				1.0	15,383.00	2,215.00	17,598.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	39,993.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
5	Post Doctoral Associates	36.0			189,118.00	27,233.00	216,351.00
2	Graduate Students		8.9	4.5	95,650.00	62,607.00	158,257.00
	Undergraduate Students						
	Secretarial/Clerical						
2	Staff Research Associate	9.0			40,335.00	17,668.00	58,003.00
9	Total Number Other Personnel					Total Other Personnel	432,611.00
						Total Salary, Wages and Fringe Benefits (A+B)	472,604.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 02-01-2024

End Date*: 01-31-2025

Budget Period: 3

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

	Funds Requested (\$)*
1 . Hamamatsu PMT 16x16 H13700 multianode array	5,700.00
2 . Vertilon 256 channel, 4 MHz custom PMT array readout electron	112,500.00

Total funds requested for all equipment listed in the attached file

Total Equipment 118,200.00

Additional Equipment: File Name:

D. Travel

	Funds Requested (\$)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	14,000.00
2. Foreign Travel Costs	

Total Travel Cost 14,000.00

E. Participant/Trainee Support Costs

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees Total Participant Trainee Support Costs 0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 1247267250000

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Regents of the University of California

Start Date*: 02-01-2024

End Date*: 01-31-2025

Budget Period: 3

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		82,169.00
2. Publication Costs		4,000.00
3. Consultant Services		
4. ADP/Computer Services		6,356.00
5. Subawards/Consortium/Contractual Costs		216,927.00
6. Equipment or Facility Rental/User Fees		20,000.00
7. Alterations and Renovations		
	Total Other Direct Costs	329,452.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	934,256.00

H. Indirect Costs		Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
Indirect Cost Type				
1 . Modified Total Direct Costs		60.5	539,009.00	326,100.00
			Total Indirect Costs	326,100.00
Cognizant Federal Agency	DHHS Cost Allocation Services, Arif M. Karim, 415-437-7820			
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	1,260,356.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		1,260,356.00

L. Budget Justification*	File Name: 29639_BudgetJustification_FINAL.pdf (Only attach one file.)
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Key Personnel

- HILLEL ADESNIK, PH.D. -- PI (1.0 SUMMER MONTHS / YEAR).

Dr. Adesnik is an associate professor of neurobiology at UC Berkeley. He will supervise all projects proposed in this application. He has over 19 years of experience working on the physiology of circuits of the mammalian central nervous system using a combination of electrophysiological, optical, and genetic approaches. He has extensive expertise with multiphoton imaging combined with neurophysiological recordings and optogenetic manipulations in awake, behaving rodents, and has been a pioneer in developing new approaches to manipulate the activity of neurons in the nervous system. The PI's detailed knowledge of these techniques will be used to initiate, and in collaboration with the co-investigators, design, manage, execute, and analyze the experiments in the proposed application.

- NA JI, PH.D. -- CO-INVESTIGATOR (0.1 SUMMER MONTHS / YEAR).

Dr. Ji is an associate professor of neurobiology at UC Berkeley. Dr. Ji is trained as a physicist and neurobiologist. Her lab develops new optical methods for *in vivo* imaging, and applies these methods to structural and functional studies of neural circuits. Dr. Ji has pioneered novel strategies for kHz voltage imaging adaptive optical correction of multiphoton imaging, achieving near-diffracted limited imaging deep into brain tissue. She will be directly involved in all aspects of the design and optimization of the imaging system.

- STEPHEN BROHAWN -- CO-INVESTIGATOR (0.1 SUMMER MONTHS / YEAR).

Dr. Brohawn is an assistant professor of neurobiology at UC Berkeley. He is an expert in membrane protein structure and function, and his labs investigates the biophysical mechanisms of ion channel gating and conduction in neurons. Dr. Brohawn will oversee the design and synthesis of opsin protein mutants for the purposes of optimizing light gating and ion conduction.

- LAURA WALLER, PH.D. -- CO-INVESTIGATOR (0.1 SUMMER MONTHS / YEAR).

Dr. Waller is an associate professor in Electrical Engineering and Computer Sciences at UC Berkeley with 17 years of experience in computational imaging and microscopy. She specializes in 3D optical imaging and phase retrieval, including nonlinear inverse problems such as those developed here. Her lab builds both hardware and software that work together to provide more efficient imaging systems that can achieve resolutions, volumes and contrast mechanism not previously possible, using relatively simple optical setups and sophisticated post-processing algorithms. She will be involved in the design and implementation of the optical system for 3D temporally focused digital holography, as well as developing novel algorithms for large scale point cloud hologram computation.

- RIKKY MULLER, PH.D. -- CO-INVESTIGATOR (1.0 SUMMER MONTHS / YEAR).

Dr. Muller, PhD is an Assistant Professor of Electrical Engineering and Computer Sciences (EECS) at the University of California, Berkeley. She is Co-director of the Berkeley Wireless Research Center (BWRC), a Core Member of the Center for Neural Engineering and Prostheses (CNEP) and an Investigator at the Chan-Zuckerberg Biohub. Her research group focuses on develop new devices for neurotechnological applications as well emerging implantable medical devices and in developing low-power, wireless microelectronic and integrated systems for neurological applications. Prof. Muller received her Bachelor's and Master's degrees in Electrical Engineering from MIT, where she worked on cellular BioMEMS devices for pathogen detection.

Other Personnel

- YI XUE, PH.D. -- POSTDOCTORAL FELLOW (ADESNIK/WALLER LAB, 8.0 CALENDAR MONTHS).

Dr. Xue received her Ph.D. in optical engineering, with a focus on computational biological imaging from MIT. Dr. Xue has extensive experience developing novel imaging modalities for biological specimens, and is an expert in all aspects of hardware and software construction for custom microscopes, particularly for multiphoton digital holography. She will help design, calibrate and optimize the holographic microscope.

- LAMIAE ABDELADIM, PH.D. -- POSTDOCTORAL FELLOW (ADESNIK LAB, 8.0 CALENDAR MONTHS).

Dr. Abdeladim received her Ph.D. from the University of Paris in optical physics and multiphoton microscopy. Dr. Abdeladim is an expert in all aspects of two-photon microscopy and has extensive experience running all-optical physiological experiments in awake mice. She will execute the in vivo animal experiments in this proposal and develop and help implement the optical design.

- **SAVITHA SRIDHARAN, PH.D. -- POSTDOCTORAL FELLOW (ADESNIK LAB, 8.0 CALENDAR MONTHS).**
Dr. Sridharan received her Ph.D. in molecular biology from the University of Texas. She has extensive experience in quantitative testing of opsins proteins with electrophysiology, having previously helped develop the ultra-potent fast opsins ChroME and ChroME2.0. She will execute in vitro imaging/electrophysiological tests combining opsin variants from the Brohawn lab and GEVLs from the Lin lab.
- **TBD, PH.D. -- POSTDOCTORAL FELLOW (JI LAB, 6.0 CALENDAR MONTHS).**
He/she will work on testing ASAP variants with FACED microscopy and comparing two photon voltage imaging quality between FACED and the holographic imaging modality.
- **TBD, PH.D. -- POSTDOCTORAL FELLOW (WALLER LAB, 6.0 CALENDAR MONTHS).**
The Postdoc will focus on the design and characterization of the imaging device and experimental testing and development of image reconstruction code.
- **KYLE TUCKER, GRADUATE STUDENT RESEARCHER (BROHAWN LAB, 4.5 ACADEMIC + 1.5 SUMMER MONTHS).**
Mr. Tucker has experience with solving EM structures of microbial opsins and their mutagenesis. He will solve the 3D structure of PsChR and other opsins in order to optimize their conductance and spectral properties.
- **TBD, GRADUATE STUDENT RESEARCHER (MULLER LAB, 4.4 ACADEMIC + 3.0 SUMMER MONTHS).**
GSRs will assist the PI, as directed, in conducting research and contribute to the drafting and dissemination of results.
- **KARTHIKA GOPAKUMAR – STAFF RESEARCH ASSOCIATE I (ADESNIK LAB, 6.0 CALENDAR MONTHS).**
Ms. Gopakumar has extensive experience with mouse colony management, animal surgery, viral injections, and post-mortem histology. She will assist the Adesnik lab in the breeding transgenic mice, and the preparation of animals for testing the multiphoton system.
- **JANINE BEYER -- STAFF RESEARCH ASSOCIATE II (ADESNIK LAB, 3.0 CALENDAR MONTHS).**
Ms. Beyer has extensive experience with AAV viral injection and validation, post-mortem histology and confocal imaging, and general lab management. She will assist in viral vector validation and administration as well as operational support.

Postdoc salaries are based on experience level.

Fringe Benefits

The University of California, Berkeley Composite Benefit Rates (CBR) have been reviewed and federally approved by the Department of Health and Human Services (DHHS) for use by all fund sources for FY21. Rates beyond June 30, 2021 are estimates and are provided for planning purposes only. Future CBRs are subject to review and approval by DHHS on an annual or bi-annual basis. Fringe benefits are assessed as a percentage of the respective employee's salary. The benefit rates are as follows:

CBR Rate Group	Approved	Projections for Planning Purposes ->				
		FY21	FY22	FY23	FY24	FY25
Academic	35.9%	35.9%	35.9%	35.9%	35.9%	35.9%
Staff	45.9%	43.8%	43.8%	43.8%	43.8%	43.8%
Limited	16.4%	14.4%	14.4%	14.4%	14.4%	14.4%
Employees with No Benefit Eligibility	5.5%	4.2%	4.2%	4.2%	4.2%	4.2%

Students	2.4%	2.6%	2.6%	2.6%	2.6%
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For more information, please see: <https://cfo.berkeley.edu/about-us/financial-planning-analysis/central-resource-management/composite-benefit-rates-cbr>

Equipment

- YEAR 1 (ADESNIK LAB):
 - a. Table optics and optomechanics: \$45,000
 - b. Meadowlark Optics HD SLM: The quoted amount is \$24,000.
 - c. Hnu Nuvu 128 EMCCD camera: The quoted amount is \$40,000.
 - d. Coherent Monaco 60W/Opera-F OPA system. The quoted amount is \$275,000.
- YEAR 2 (MULLER LAB):
 - a. Memscap: A purchase of fabricated wafers of microelectromechanical mirrors is planned for year 2 of the project. The quoted amount for such a purchase as of 4/23/2021 is \$67,511.
 - b. T-Micro: A purchase of assembled spatial light modulators from T-Micro is planned for year 2 of the project. The quoted amount for this item is \$12,450.
- YEAR 3 (ADESNIK LAB):
 - a. Hamamatsu PMT 16x16 H13700 multianode array \$5,700
 - b. Vertilon 256 channel, 4 MHz custom PMT array readout electronics \$112,500

Travel

- DOMESTIC: We are requesting \$14,000 for travel to attend domestic conferences and research meetings, at \$2,000 person. Cost will consist of airfare, conference registration fees, lodging, meal and incidentals expenses.

Other Direct Costs

- MATERIALS AND SUPPLIES: We are requesting \$96,470 / \$90,651 / \$82,169 in materials and supplies for surgery supplies, preparation of animals for experiments, histology supplies for post-mortem brain histology, AAV viral preparations (UPenn Vector Core external services), molecular cloning supplies including oligos for performing two-site mutagenesis. Mouse animal care costs (Adesnik and Ji lab, UC Berkeley per diem at \$0.69 per cage), we expect enough cages for testing and validating the system in the visual cortex.
- PUBLICATIONS: Amount varies according to journal, number of figures, and total publications per year. We are requesting \$2,000 for Year 1, \$4,000 for Year 2 & 3.
- COMPUTING SERVICES: \$6,356/year requested for computing charges in the Brohawn and Waller labs.
- EQUIPMENT/RENTAL: LASER SERVICE CONTRACTS: \$20,000/year for two lasers and the OPA system.
- SUBCONTRACTS: STANFORD UNIVERSITY: Funds requested for a subaward to Stanford University. Please see subaward budget and justification for further details.

Indirect Costs

Indirect costs are based on University negotiated rates with the cognizant federal authority and are applied at a rate of 59.0% from 07/01/2020–06/30/2021 and increasing to 60.5% from 07/01/2021–06/30/2022. Indirect costs are applied using the Modified Total Direct Cost (MTDC) formula, per rate agreement dated October 24, 2019. Modified total direct costs exclude equipment, capital expenditures, charges for patient care, student tuition remission, rental costs of off-site facilities, scholarships, and fellowships, participant

support costs and the portion of each subgrant and subcontract in excess of \$25,000. For more information, please see: <http://www.spo.berkeley.edu/policy/fa.html>. The rates after July 1, 2022 are provisional and subject to change based upon our updated federally negotiated indirect cost rate agreement.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	117,973.00
Section B, Other Personnel	1,268,727.00
Total Number Other Personnel	27
Total Salary, Wages and Fringe Benefits (A+B)	1,386,700.00
Section C, Equipment	582,161.00
Section D, Travel	42,000.00
1. Domestic	42,000.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	997,138.00
1. Materials and Supplies	269,290.00
2. Publication Costs	10,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	19,068.00
5. Subawards/Consortium/Contractual Costs	638,780.00
6. Equipment or Facility Rental/User Fees	60,000.00
7. Alterations and Renovations	0.00
8. Other 1	0.00
9. Other 2	0.00
10. Other 3	0.00
Section G, Direct Costs (A thru F)	3,007,999.00
Section H, Indirect Costs	989,947.00
Section I, Total Direct and Indirect Costs (G + H)	3,997,946.00
Section J, Fee	0.00
Section K, Total Costs and Fee (I + J)	3,997,946.00

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Enter name of Organization: Stanford University

Start Date*: 02-01-2022

End Date*: 01-31-2023

Budget Period: 1

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1 .	Michael	Z.	Lin		Subaward PI	199,300.00	1.2			19,930.00	5,780.00	25,710.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	25,710.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar	Months	Academic	Months	Summer	Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
1	Post Doctoral Associates		12.0					66,388.00	16,663.00	83,051.00
	Graduate Students									
	Undergraduate Students									
	Secretarial/Clerical									
1	Total Number Other Personnel							Total Other Personnel	83,051.00	
								Total Salary, Wages and Fringe Benefits (A+B)	108,761.00	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Organization: Stanford University

Start Date*: 02-01-2022

End Date*: 01-31-2023

Budget Period: 1

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

Funds Requested (\$)*

Total funds requested for all equipment listed in the attached file

Total Equipment 0.00

Additional Equipment: File Name:

D. Travel

Funds Requested (\$)*

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)

2. Foreign Travel Costs

Total Travel Cost 0.00

E. Participant/Trainee Support Costs

Funds Requested (\$)*

1. Tuition/Fees/Health Insurance

2. Stipends

3. Travel

4. Subsistence

5. Other:

Number of Participants/Trainees

Total Participant Trainee Support Costs

0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Organization: Stanford University

Start Date*: 02-01-2022

End Date*: 01-31-2023

Budget Period: 1

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		24,000.00
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
	Total Other Direct Costs	24,000.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	132,761.00

H. Indirect Costs		Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
Indirect Cost Type				
1 . Modified Total Direct Costs		57.4	132,761.00	76,205.00
			Total Indirect Costs	76,205.00
Cognizant Federal Agency				
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	208,966.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		208,966.00

L. Budget Justification*	File Name: 29639_Stanford_BudgetJustification.pdf (Only attach one file.)
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Enter name of Organization: Stanford University

Start Date*: 02-01-2023

End Date*: 01-31-2024

Budget Period: 2

A. Senior/Key Person

	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Michael	Z.	Lin		Subaward PI	199,300.00	1.2			19,930.00	5,780.00	25,710.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	25,710.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
1	Post Doctoral Associates	12.0			68,379.00	17,163.00	85,542.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Total Number Other Personnel				Total Other Personnel		85,542.00
					Total Salary, Wages and Fringe Benefits (A+B)		111,252.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Organization: Stanford University

Start Date*: 02-01-2023

End Date*: 01-31-2024

Budget Period: 2

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

Funds Requested (\$)*

Total funds requested for all equipment listed in the attached file

Total Equipment

0.00

Additional Equipment: File Name:

D. Travel

Funds Requested (\$)*

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)

2. Foreign Travel Costs

Total Travel Cost

0.00

E. Participant/Trainee Support Costs

Funds Requested (\$)*

1. Tuition/Fees/Health Insurance

2. Stipends

3. Travel

4. Subsistence

5. Other:

Number of Participants/Trainees

Total Participant Trainee Support Costs

0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Organization: Stanford University

Start Date*: 02-01-2023

End Date*: 01-31-2024

Budget Period: 2

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		24,000.00
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
	Total Other Direct Costs	24,000.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	135,252.00

H. Indirect Costs		Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
Indirect Cost Type				
1 . Modified Total Direct Costs		57.4	135,252.00	77,635.00
			Total Indirect Costs	77,635.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	212,887.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		212,887.00

L. Budget Justification*	File Name: 29639_Stanford_BudgetJustification.pdf (Only attach one file.)
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Enter name of Organization: Stanford University

Start Date*: 02-01-2024

End Date*: 01-31-2025

Budget Period: 3

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Michael	Z.	Lin		Subaward PI	199,300.00	1.2			19,930.00	5,780.00	25,710.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	25,710.00
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B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
1	Post Doctoral Associates	12.0			70,431.00	17,678.00	88,109.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Total Number Other Personnel				Total Other Personnel		88,109.00
					Total Salary, Wages and Fringe Benefits (A+B)		113,819.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Organization: Stanford University

Start Date*: 02-01-2024

End Date*: 01-31-2025

Budget Period: 3

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

Funds Requested (\$)*

Total funds requested for all equipment listed in the attached file

Total Equipment

0.00

Additional Equipment: File Name:

D. Travel

Funds Requested (\$)*

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)

2. Foreign Travel Costs

Total Travel Cost

0.00

E. Participant/Trainee Support Costs

Funds Requested (\$)*

1. Tuition/Fees/Health Insurance

2. Stipends

3. Travel

4. Subsistence

5. Other:

Number of Participants/Trainees

Total Participant Trainee Support Costs

0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 0092142140000

Budget Type*: Project Subaward/Consortium

Organization: Stanford University

Start Date*: 02-01-2024

End Date*: 01-31-2025

Budget Period: 3

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		24,000.00
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
	Total Other Direct Costs	24,000.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	137,819.00

H. Indirect Costs		Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
Indirect Cost Type				
1 . Modified Total Direct Costs		57.4	137,819.00	79,108.00
			Total Indirect Costs	79,108.00
Cognizant Federal Agency				
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	216,927.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		216,927.00

L. Budget Justification*	File Name: 29639_Stanford_BudgetJustification.pdf (Only attach one file.)
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification

As of 9/30/2020 the indirect cost rate is the on-campus rate of 57.4% and is based on a Modified Total Direct Cost Base (MTDC). MTDC excludes tuition, animal care, equipment and patient care. The fringe benefit rate is 29.0% for faculty/staff, 25.1% for postdoctoral affiliates, 5.3% for graduate students, and 8.1% for temporary/casual personnel. Salaries are capped at the current NIH level of \$199,300.

Per our negotiated rate agreement with the Office of Naval Research, the budgeted salary amount for staff includes 8.7% vacation accrual/disability sick leave (DSL) for exempt and non-exempt employees. This amount does not exceed total salary. The vacation accrual/DSL rates will be charged at the time of the salary expenditure. No salary will be charged to the award when the employee is on vacation, disability or worker's compensation.

Key Personnel

Michael Lin, MD, PhD, Co-Investigator. (1.2 CM) Dr. Lin will oversee the generation of improved red and yellow ASAPs. He will supervise the postdoctoral fellow Dr. Zhao and help with experimental design and analysis.

Other Personnel

Yufeng Zhao, Postdoctoral Fellow. (12 CM) Dr. Zhao perform all experiments involving improving red and yellow ASAPs by high-throughput screening. Dr. Zhao has extensive experience in library cloning, screening, and fluorescent indicator development from his PhD training in chemistry with Robert Campbell.

Supplies

We are requesting \$24,000 per year in supplies. The largest costs are for oligos for performing two-site mutagenesis, which cost about \$1600 per pair of 384-well plates. Supplies also include other molecular biology reagents, plasticware, glass-bottom 384-well plates for performing the screen, and cell culture media.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	77,130.00
Section B, Other Personnel	256,702.00
Total Number Other Personnel	3
Total Salary, Wages and Fringe Benefits (A+B)	333,832.00
Section C, Equipment	0.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	72,000.00
1. Materials and Supplies	72,000.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other 1	0.00
9. Other 2	0.00
10. Other 3	0.00
Section G, Direct Costs (A thru F)	405,832.00
Section H, Indirect Costs	232,948.00
Section I, Total Direct and Indirect Costs (G + H)	638,780.00
Section J, Fee	0.00
Section K, Total Costs and Fee (I + J)	638,780.00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	1,106,961	812,942	855,148	0	0	2,775,051

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? Yes No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

Yes No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

Yes No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount (\$) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? Yes No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? Yes No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: Yes No

If the answer is "Yes" then please answer the following:

*Previously Reported: Yes No

6. Change of Investigator/Change of Institution Section

Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Introduction

1. Introduction to Application
(for Resubmission and Revision applications)

Research Plan Section

2. Specific Aims 29639_SpecificAims_FINAL.pdf
3. Research Strategy* 29639_ResearchStrategy_FINAL.pdf
4. Progress Report Publication List

Other Research Plan Section

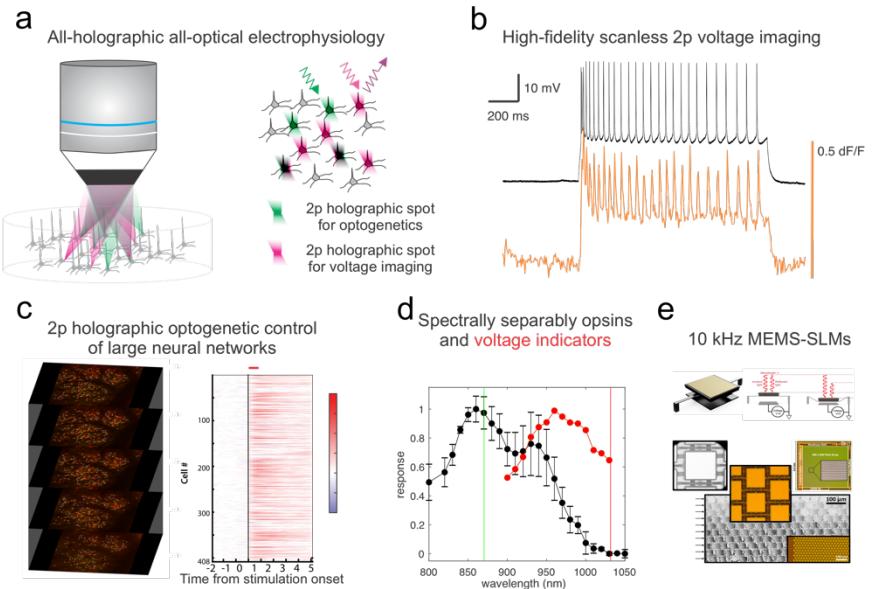
5. Vertebrate Animals 29639_VertebrateAnimals_FINAL.pdf
6. Select Agent Research
7. Multiple PD/PI Leadership Plan
8. Consortium/Contractual Arrangements 29639_Consortium_FINAL.pdf
9. Letters of Support 29639_Letters_FINAL.pdf
10. Resource Sharing Plan(s) 29639_ResourceSharing_FINAL.pdf
11. Authentication of Key Biological and/or Chemical Resources 29639_AuthKeyResources_FINAL.pdf

Appendix

12. Appendix

SPECIFIC AIMS

Achieving a detailed understanding of the neural codes of sensation, action and cognition requires technologies that can both sample and perturb neural activity with millisecond precision and cellular resolution across large populations of neurons. To address this need, we will develop an all-optical all-holographic two-photon microscope that can both measure and manipulate neuronal action potentials and subthreshold activity from large neural networks in the brains of behaving animals. The system will exploit multispectral temporally focused computer generated holography. This new form of two-photon holographic microscopy will enable simultaneous optogenetic control and voltage imaging of large user-defined ensembles of neurons, with a target of 320 unique neurons *per millisecond*. The system projects cell-size spots of temporally focused two-photon excitation light at two different wavelengths – one for imaging the genetically encoded voltage sensor and one for controlling neural activity optogenetically, in 3D. The two holographic optical paths will be independent and update at up to 10 kilohertz (kHz) speed. The major advantage of holographic imaging over other approaches is that the user can select the specific ensemble of neurons to simultaneously image and perturb and these neurons can be distributed anywhere in the addressable volume of the microscope. To optimize the approach, we will engineer new spectrally separable red-shifted two-photon excitable GEVs and blue-shifted microbial opsins that are sensitive and potent. To achieve sub-millisecond voltage sampling and optogenetic control speeds, we will build and optimize **10 kHz** frame-rate MEMS-based spatial light modulators that will dramatically scale up the temporal rate and multiplexing of the read/write process. Finally, we will develop task-optimized computational imaging algorithms to sample the voltage of many neurons holographically by leveraging the ultra-fast SLM update rates. The development of an all-holographic read/write microscope will permit neuronal voltage imaging *and* perturbation on spatial and temporal scales that are currently well beyond reach. This new technology could substantially accelerate systems neuroscience research.



Core components of the project. a) Schematic of the new microscope technology. b) High performance 2p voltage imaging. c) Large-scale, cellular resolution optogenetics. d) New spectrally separable opsins and voltage indicators. e) Novel, ultra-fast MEMS-SLMs.

Aim 1: Develop holographic voltage imaging 2p microscopy

- 1.1 Test and validate scanless 2p voltage imaging.
- 1.2 Build, calibrate and validate a holographic voltage imaging 2p microscope *in vivo*.
- 1.3 Develop novel task-optimized computational imaging approaches for source extraction.
- 1.4 Benchmark holographic imaging against kHz scanning (FACED) 2p imaging.

Aim 2: Develop optimized ASAP4 variants and potent ultra-blue opsins for spectrally separable imaging and optogenetics

- 2.1 Develop sensitive and photo-stable red-shifted ASAP sensors.
- 2.2 Engineer ultra-potent, highly blue-shifted opsins for spectrally separable 2p imaging and optogenetics.

Aim 3: Develop a dual holographic two-photon microscope for simultaneous optogenetics and voltage imaging with cellular resolution and millisecond precision

- 3.1 Build and validate a multi-spectral holographic two-photon microscope for simultaneous voltage imaging and two-photon optogenetics.
- 3.2 Build and integrate ultra-fast (10 kHz) MEMS-SLMs for both imaging and optogenetics to substantially increase the speed and throughput of the system.
- 3.3 Pilot spatiotemporal multiplexing of holographic paths to increase system throughput.

Significance: Advances in cellular resolution imaging and perturbation have transformed our understanding of the neural codes of sensation, cognition, and behavior. In particular, optical methods that leverage genetically encoded sensors and actuators of neural activity have gained widespread adoption because they provide direct access to genetically defined cell types and they permit dense sampling and perturbation of neural circuits with cellular resolution. Approaches that combine two-photon (2p) calcium imaging with 2p optogenetics (1)(2)(3)(4)(5) have yielded key new insights into neural coding because they can causally relate features of neural activity to network dynamics and behavior(6)(7)(8)(9)(10)(11)(12). However, since calcium imaging has low temporal resolution(13), only indirectly measures spiking activity, and provides no access to subthreshold voltage responses, these systems cannot probe fundamental features of the neural code that occur on faster timescales (14)(15)(16)(17) or are contained in subthreshold potentials(18)(19). To overcome this technical gap, **we will develop an optical approach that both measures and manipulates the voltage of large numbers of neurons with millisecond precision and cellular resolution in behaving animals.** This new form of all-optical electrophysiology will require new microscope systems design that exploit the properties of voltage imaging, but will facilitate major discoveries by bridging the cellular, circuit and computational levels of analysis.

The new system will combine kilohertz (kHz) 2p voltage imaging with kHz 2p optogenetics. Voltage imaging can directly report supra- and sub-threshold activity with millisecond resolution from specific cell types and do so in a densely labeled neural circuit(20). 2p optogenetics can provide cellular resolution control over neural activity with high temporal precision(2)(6) (21)(22). Although there has been some progress combining optogenetics and voltage imaging in cell culture(23) or the topmost layer of cells in the mammalian circuits with visible light excitation(24)(25), a system that achieves read/write control non-invasively but deeper in the brain is lacking. To achieve this, we must develop a 2p excitation approach, scale the throughput to image large populations of neurons, extend it to operate in three dimensions (3D), and achieve kHz speed.

To address all these challenges, we will develop and validate an **all-holographic** 2p excitation platform. It relies on multispectral temporally-focused three-dimensional (3D) wavefront shaping (Fig. 1) which will enable simultaneous illumination of user-defined ensembles of neurons in 3D with cell-size spots of 2p excitation light(2)(21)(26) at two different wavelengths – one for imaging a voltage sensor and one for exciting an optogenetic actuator. The two holographic optical paths will be independent and update at 10 kHz speed by using novel micro-electromechanical system spatial light modulations (MEMS-SLMs)(27).

Holographic imaging (28)(29) is particularly advantageous for neural activity monitoring because the user can target light to specific groups of neurons (among a much larger population) based on a particular physiological feature, such as their responsiveness to a specific stimulus or during a specific behavioral task. In many brain regions only a sparse, often distributed subset of neurons is active under any given condition (30)(31)(32)(33)(34)(35). In these situations, conventional raster scanning is a non-economical use of imaging time and laser power, while holographic sampling can be maximally efficient since it can specifically illuminate the neurons of greatest interest and sample their activity at speeds limited only by the frame rate of the sensor.

To enable all-optical, all-holographic electrophysiology we must also engineer or identify new voltage sensors and new opsins that are *spectrally separable* but also sensitive and potent. We will thus develop new red-shifted 2p-excitable genetically encoded voltage indicators (GEVIs) that are optimally suited to this task. At the same time, we will engineer ultrapotent highly blue-shifted optogenetic tools that are spectrally separable from the GEVI in the 2p regime so as to eliminate any unwanted optogenetic depolarization by the voltage imaging light. Finally, we will combine all these aforementioned advances into **one integrated system that can sample and control the membrane voltage of hundreds of neurons simultaneously at kHz speed**.

A critical design feature in our 2p holographic approach is that at each instant in time the femtosecond holograms we will use will be highly sparse in space. Sparsity will enable high spatial precision of illumination

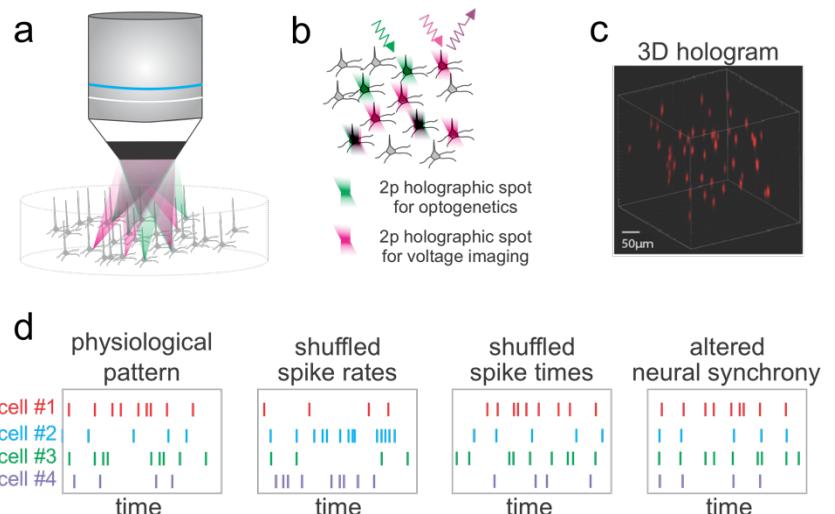


Figure 1: All holographic 2p electrophysiology. a) Schematic of a two-color holographic microscope. b) Cells are targeted with different wavelengths for 2p imaging and optogenetics. c) A 2p hologram composed of many spots to illuminate neurons in 3D. d) A strategy to address neural codes with the new technology by rewriting activity patterns.

and will simplify the fluorescence readout process when light scatter in brain tissue may mix signals from different cells in the image. By rapidly interleaving sparse holograms at 10 kHz for both optogenetics and voltage imaging we can maintain spatial precision while increasing the scale of the system's sampling abilities. Together, we expect that these innovations will yield a platform for ultra-fast, large-scale read/write control of neural voltage in the intact mammalian brain to address major outstanding questions in neuroscience. For example, one could measure a physiological pattern of network activity with millisecond precision and then replay altered versions of the pattern into the brain to assess the impact on circuit dynamics and behavior (**Fig. 1d**). Such experiments could address how precise sequences of spikes, the synchrony of spikes, or the absolute rate of spikes in different neurons contribute to sensation, motor action and the generation of synaptic plasticity. To help disseminate this technology, we will work with existing commercial vendors of holographic microscopes (Bruker, Scientifica, 3i, Neurolabware) to enable simultaneous holographic 2p optogenetics and holographic 2p imaging.

Background: High-speed voltage imaging is challenging because the signal change occurs only in the thin plasma membrane limiting the amount of useful fluorescent sensor, while the brief sampling periods require bright sensors and very sensitive measurement devices(36)(20). For all-optical electrophysiology, an additional challenge is that the voltage sensor and the optogenetic actuator must be spectrally separable such that the excitation light for imaging does not perturb the cells of interest. Recent advances in both GEVs and optical approaches for sampling their fluorescence have permitted direct optical measurements of the action potentials and sub-threshold potentials of small ensembles of neurons in the intact brain(25)(37)(38). Nevertheless, critical drawbacks to both existing voltage imaging technologies and available GEVs constrain their application. Microbial-opsin based sensors have many favorable qualities but lose voltage sensitivity under pulsed 2p excitation 2p excitation(39)(40). This limits their applicability to very superficial brain structures, to more sparsely labeled samples, or requires implanted optics (e.g., GRIN lenses). 2p excitable GEVs and genetically targetable dyes rely on coupling a bright fluorescent protein to a voltage-sensing domain(41)(42), or conjugating a synthetic voltage sensing dye to a genetically targeted membrane anchor(43)(44). Among GEVs, the most recent ASAP family variants exhibit fast kinetics, high brightness, and suitable photo-stability to adequately report action potentials in typical neurobiological experiments(45).

Several groups pioneered 2p voltage imaging approaches with high spatial and temporal precision in brain slices and *in vivo* using various sensors such as ArcLight(40), VSFPs(42), and ASAPs(46). A comparative evaluation in 2020 found that ASAP3 exhibited the largest fluorescence changes and the fastest kinetics among these and other GEVs(47). Recently, two teams leveraged the improved sensor ASAP3 with either random access 2p microscopy (U-LOVE)(45) or ultra-fast multi-focal imaging (FACED)(48) (**Fig. 2**) to demonstrate the direct sampling of neuronal spiking and sub-threshold activity in awake mice. This establishes ASAP3 as a useful sensor for 2p voltage imaging, but in both studies the total number of simultaneously sampled neurons was low (1-15). Ultimately, these point scanning approaches are throughput-limited by the need to physically steer the imaging beam(s) across the sample neurons. Clever alternative scanning methodologies with engineered point-spread functions (such as advanced light sheet(49)(50), SLAP(51), or reverberation microscopy(52) and other approaches(53)(54)) can increase the speed but have not yet achieved kHz-rate 2p voltage imaging *in vivo*. But more importantly, since the relevant physiological information only comes from a small subset of the total points in the imaging volume, a sparse, scanless, parallel illumination technique could overcome all of these limitations.

We propose that computer generated holography (CGH) solves these challenges. CGH is a parallel illumination technique that a growing number of groups have used for optogenetic control(28)(55)(56) and imaging(57)(29)(28)(58)(59). It can achieve high resolution 2p excitation across many neurons at a time in the intact brain. Temporally focusing the 2p illumination spots provides high axial precision in all three dimensions(26)(60), such as via the technology '3D-SHOT' (three dimensional scanless holographic optogenetics with temporal focusing)(21). We previously developed 3D-SHOT to control large neural ensembles optogenetically up to several hundred cells at a time, see **Fig. 3**(2)(61), but a similar wavefront engineering

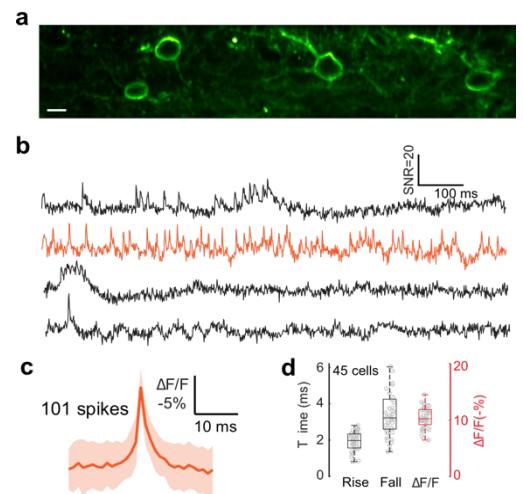


Figure 2: 2p voltage imaging with ASAP GEVs.
a) Average image during kHz FACED scanning of ASAP3-expressing neurons *in vivo*. b) Spontaneous voltage activity in four example neurons. c) Average of 101 detected spikes from the second neuron in b). d) Kinetics and $\Delta F/F$ of detected spikes(46).

scheme can be applied to excite fluorescent voltage sensors whose emission can be collected by parallel detectors such as cameras or arrays of photo-multiplier tubes (PMTs). 3D-SHOT thus represents a random access but parallel imaging approach whose main strengths are focusing light exactly where it could be maximally informative. With spectrally separable optogenetic actuators and voltage sensors one could use two parallel 3D-SHOT systems with two different wavelengths to simultaneously measure *and* control neuronal voltage with cellular resolution and millisecond precision. This project is aimed at developing the proof-of-concept of such a system. We will test and validate the dual-holographic system for kHz read/write control of neural activity, and we will engineer microbial opsins that are spectrally separable from the GEVI. Our goal is to demonstrate the simultaneous recording and stimulation of several dozen to several hundred neurons simultaneously with kHz speed in the neocortex of awake, behaving mice. A system with these capabilities could address a wide array of important problems in brain function and disease that cannot be resolved by any other means.

Comparison to high-throughput electrophysiology: 2p voltage imaging has several advantages over more established high-throughput voltage measurement approaches like high-channel count multi-electrode arrays, but also some weaknesses. The system we will develop can sample densely from a targeted volume of brain tissue, directly identify specific cell types (when they express a fluorescent protein), control the neural activity of the same group of cells via cellular-resolution optogenetics, measure subthreshold voltage potentials and repeatedly sample, and perturb the same identified groups of neurons chronically over time. Its main limitation is that it will be restricted to optically accessible areas of the brain, although future development can extend it by combining with implanted relay optics such as GRIN lenses(62) or microprisms. In some cases future nanophotonic probes will be more suitable(63).

Innovation: The primary innovation is the development of a new technological platform for ultra-fast high-throughput sampling and optogenetic control of neuronal membrane voltage from large populations of neurons *in vivo* with cellular precision. No existing technology achieves this, and it could open up major new avenues of investigation in neural coding, computation, plasticity and behavior. The specific innovations include: 1) the first development and demonstration of 2p holographic voltage imaging; 2) the first 2p all-optical electrophysiological platform for supra- and sub-threshold measurement and control with kHz resolution; 3) novel ultra-fast (10 kHz) MEMS-SLMs for optogenetics and voltage imaging; 4) new bright, red-shifted, fast and 2p excitable GEVIs with high photo-stability and sensitivity; 5) novel, highly blue-shifted ultrapotent blue microbial opsins; 6) new computational imaging strategies for optimally sampling and demixing the voltage signals from sparsely, holographically illuminated neuronal sources.

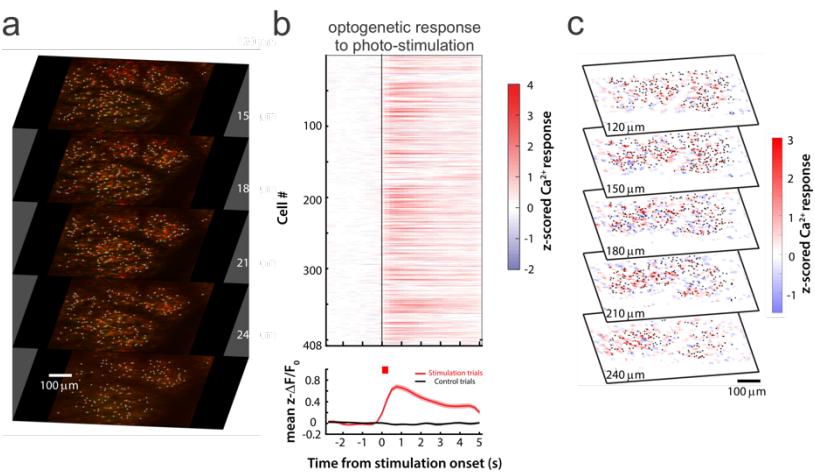


Figure 3: 3D-SHOT can illuminate and control large neural networks in 3D. a) A 5-plane 2p image from mouse neocortex of neurons co-expressing an opsin (ChroME2.0) and GCaMP6. b) Calcium response plot of hundreds of neurons that were holographically stimulated *simultaneously* with 2p excitation. c) Normalized calcium response of the activated neurons across the five imaging planes.

Team: Our highly interdisciplinary team combines the necessary expertise in neurophysiology, optics, molecular engineering, electrical engineering, and computation to develop and validate the proposed technology. **The Adesnik lab** will lead the collaboration and leverage its prior success developing and optimizing 3D-SHOT (with the Waller and Ji labs), and engineering high performance optogenetic proteins (with the Brohawn Lab). It has the optical and neurophysiological expertise to build the proposed microscope and validate it in the intact brain with simultaneous imaging and ground truth electrophysiology. **The Lin lab** developed ASAP sensors and will develop red-shifted high performance ASAP variants that will be spectrally separable from microbial opsins for optogenetics. **The Brohawn lab**, whose expertise is protein structure, has collaborated with the Adesnik lab to rationally design high performance opsins and has obtained the first electron microscopic structures of opsin proteins. They will extend this work to develop highly potent ultra-blue opsins. **The Ji lab** developed 2p FACED technology which achieves kHz-raster scanning imaging of ASAP sensors *in vivo*. They will help design and validate the new holographic optical systems and GEVIs and benchmark performance against FACED. **The Muller lab** will develop and fabricate MEMS-based spatial light modulators (SLMs) that will provide a ~20-fold

improvement in refresh rate over existing SLMs to increase the speed and throughput of voltage imaging and optogenetics. **The Waller lab** previously collaborated with the Adesnik lab to develop 3D-SHOT and other 3D computational imaging and stimulation technologies for brain circuits. They will help design and optimize the optical system as well as the novel computational algorithms that will be able to sample and separate the fluorescent signals of the holographically excited neurons during parallel detection.

Aim 1: Develop holographic voltage imaging 2p microscopy

Aim 1.1: Test and validate scanless 2p voltage imaging: First, we will demonstrate that scanless 2p excitation of ASAP voltage sensors can report both action potentials and subthreshold potentials. We recently developed ASAP4.4, which is a new GEVI with significantly improved photo-stability, positive going fluorescence changes for depolarizations, and importantly, a slight red-shift in its excitation and emission spectrum, all desirable features for 2p voltage imaging. We will conduct all experiments in this proposal on both sexes of mice and analyze the data for sex-specific differences. We expressed soma-targeted ASAP4.4 in L2/3 pyramidal cells (PCs) via *in utero* electroporation in mice. In brain slices, we made whole-cell recordings to both monitor voltage and inject current to generate simulated EPSPs and spikes. Pilot data from several cells is shown in **Fig. 4a**. We injected a step pulse of current into L2/3 PCs to drive depolarization and to generate spikes and excited ASAP4.4 with a ~12.5 μm wide, non-scanned laser spot from a 10 MHz, 1040 nm laser (Spectra Physics Femtotrain, total spot energy ~100-300 mW) over the soma and captured fluorescence at 200-500 Hz with an sCMOS camera. Spikes were easily visible as large fluorescence changes of nearly 0.3 dF/F (**Fig. 4a**). The sub-threshold response was also easily observable on single trials. This pilot data demonstrates that ASAP4.4, combined with scanless 2p excitation, provides the needed signal to readily detect spikes and measure sub-threshold depolarizations on single trials. By using higher performance detectors (a specialized ultra-low noise EMCCD or a PMT array) we expect to obtain even better measurements of voltage changes at higher frame rates. We will systematically determine the threshold of the system for detecting small subthreshold events (by simulating EPSPs of various sizes with the patch electrode) on single trials. Next, we will perform similar simultaneous patch-clamp and 2p voltage imaging experiments *in vivo* and correlate signal quality with depth of the recorded cell from the brain surface.

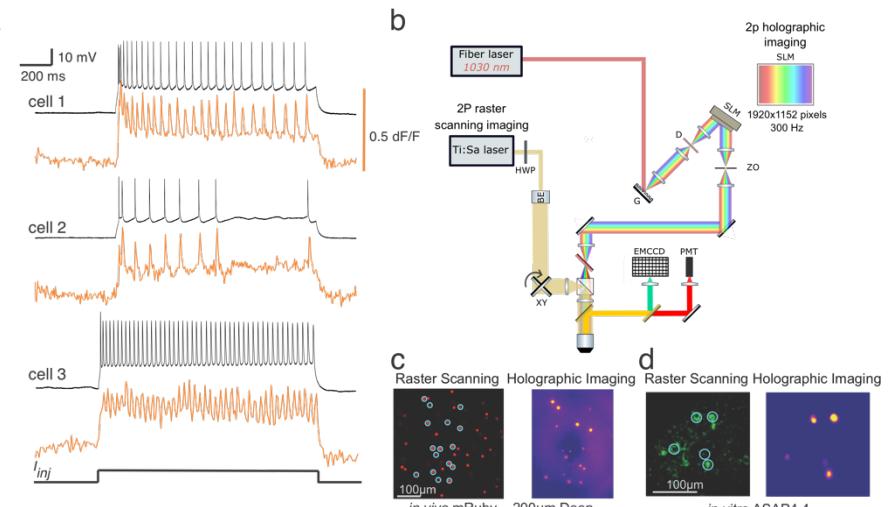


Figure 4: High-fidelity voltage sensing by ASAP4.4 with scanless 2p excitation.
 a) Three example neurons whose voltage was simultaneously recorded with a patch electrode and scanless 2p excitation of ASAP4.4 (@1040nm). Black: electrophysiology; orange: voltage imaging. b) Design schematic of the holographic microscope. Rainbow coloring indicates temporal focusing. c) Left: scanning 2p image of mRuby3-expressing neurons. Circled cells are selected for holographic illumination; right: camera image during holographic imaging. d) As in c) but for 5 ASAP4.4-expressing neurons.

Aim 1.2: Build, calibrate and validate a holographic voltage imaging 2p microscope *in vivo*. The initial system will employ a high energy fiber laser (Amplitude Satsuma 20W, 1030 nm, repetition rate tunable from 1-20 MHz) and a 3D-SHOT pathway for holographic illumination of many spots in 3D (**Fig. 4b**). The system will also include a conventional raster-scanning imaging path for structural imaging of the expressing cells and for calcium imaging (Coherent Chameleon). 3D-SHOT uses 3D CGH to generate axially resolved discs of 2p excitation light that can be arbitrarily distributed across the entire addressable volume of a conventional 2p microscope (~1x1x0.3 mm). We will illuminate groups of neurons with 3D-SHOT as we have done previously for optogenetic stimulation(2), but here to excite ASAP4.4. An example image with 20 neurons expressing mRuby3 illuminated holographically *in vivo* is shown in **Fig. 4c**. Despite light scatter, the targeted neurons are clearly resolvable in the camera image. An image of 5 holographically excited ASAP4.4 neurons is also shown in Fig. 4c. We will collect emission on a fast, high quantum efficiency, ultra-low read-noise EMCCD camera (Nüvü Hnū, 1.4kHz full frame rate).

Aim 1.3: Develop novel task-optimized compressed sensing imaging approaches for 3D source extraction: A key issue for parallel excitation/parallel detection 2p methods is de-mixing fluorescence emission from different cells on the sensor due to scatter and/or defocus. First, we will mitigate mixing by using soma-targeted ASAP4.4 (via

a Kv2.1 c-terminal tag) to minimize dendritic expression. Second, and perhaps more importantly, we will ensure high sparsity in each illumination hologram. Although 3D-SHOT can generate many spots at a time, keeping the spot number low and the spots far apart in the brain will reduce the mixing of emitted photons from different neurons onto individual pixels of the sensor. To test these notions, we conducted simulations of holographic illumination in a $1 \times 1 \times 0.1$ mm volume, using a $200 \mu\text{m}$ scattering length for cortical tissue and the simulating the impact of defocus using a conventional 1.0NA objective (**Fig. 5a,b**). The simulation implies sparsity is critical to mitigate overlap in the image. While this illumination geometry constraint is not prohibitively limiting, we ultimately plan to overcome the need for it by computational optimization of the image reconstruction (**Fig. 5c**), by ultrafast interleaving of phase masks (see Aim 3), and potentially through ultra-fast de-scanned detection. Computational algorithms (e.g., non-negative matrix factorization) for source separation have previously proven useful for calcium imaging data(64)(65) and are especially effective in situations with sparse illumination spots, as in our case. We will set up the 3D image reconstruction problem as a constrained optimization in which we have prior knowledge of the illumination spot positions, and the temporal signatures of the signals. Both of these priors drastically reduce the ill-posedness of the reconstruction problem, thus enabling us to solve for the emission intensity at each illuminated spot, even with significant scattering and/or defocus. Further, we will explore novel ideas for exploiting the high speed of the detectors to use temporal coding as a means for separating signals from nearby illumination spots. The design of the multi-spot illumination patterns will be optimized in an end-to-end fashion(66) for optimal performance. For example, we expect that superficially in the brain we could excite more neurons at a time at higher density due to reduced scatter, while as we go deeper, we will need to reduce the number of spots to ensure proper source separation.

To test this system and validate it against ground truth data, we will make whole-cell patch clamp recordings of ASAP4.4-expressing neurons in mouse primary visual cortex (V1) at different depths *in vivo* and illuminate the patched neurons as well as 5-20 other neurons simultaneously. To generate cortical activity, we will present drifting gratings of various orientations, contrasts, and sizes to the contralateral eye. We will compare the signal fidelity obtained from voltage imaging from the patched neuron and compare it to the electrophysiological data as a function of the number and density of cells illuminated and the depth from the brain surface. This will quantify the performance limits of the initial kHz voltage imaging system.

Aim 1.4: Benchmark holographic imaging against kHz scanning (FACED) 2p imaging. We previously developed kHz voltage imaging of ASAP3 with FACED 2p imaging(48). Here we will exploit FACED to benchmark the new holographic imaging technology as well as the new voltage sensors we will develop (Aim 2). We will add a holographic imaging path to our FACED microscope. To compare performance of FACED and holography, we will make whole-cell recordings from ASAP4.4-expressing neurons in brains slices and measure the quality of optically measured action potentials and sub-threshold potentials using these two schemes (on different trials).

Caveats and alternative approaches: Signal-to-noise ratio (SNR): our pilot data shows that exciting ASAP4.4 at 1040 nm (10MHz) with ~ 200 mW energy is sufficient to drive enough signal for accurately measuring voltage even with a conventional sCMOS camera. *In vivo* conditions will make this more challenging due to scatter and absorption, as will scaling up to kHz speeds owing to shorter acquisition times. Several key modifications to our first microscope should, when combined, substantially increase our sensitivity, including: leveraging an EMCCD with extremely low read noise (or PMT arrays, see below), exciting at 1 MHz repetition rate (higher pulse energy per average power), and optimizing sensor expression levels (while avoiding potentially toxic levels). In subsequent aims we will develop and employ red-shifted ASAPS that will be more efficiently excited at 1030 nm and beyond, and whose emission (>590 nm) is less scattered and absorbed by brain tissue. However, if excitation at 1030 nm does not prove sufficient, we can employ a tunable high energy system by pumping an optical parametric amplifier with the 1030 nm laser and excite at the GEVI's preferred wavelength.

Brain heating: In our pilot data we used up to 200 mW of laser energy per cell, but this was under what are likely to be highly sub-optimal conditions (wavelength, laser repetition rate, camera sensor). We expect to use

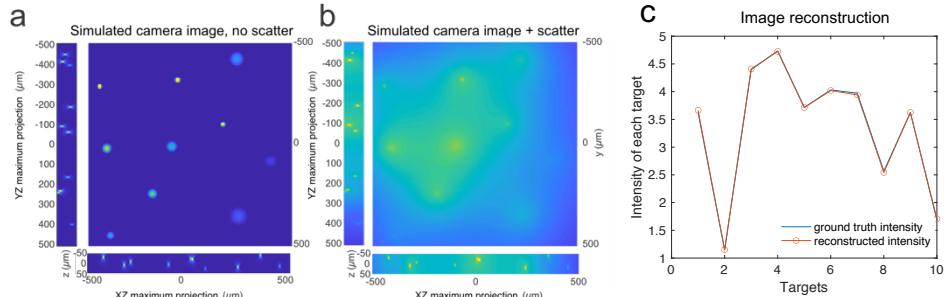


Figure 5: Simulation and reconstruction of holographic imaging data. a) Simulated camera image (128x128 pixels) for 10 holographic foci in 3D without scatter. b) As in a) but with scatter + Poisson noise. c) Reconstruction of each focus' intensity from panel b) using a fast iterative shrinkage algorithm (FISTA), and comparison to the ground truth.

substantially lower power per cell when our system is fully optimized and ideally aim to use 10-fold lower power or even less when using optimized sensors and collection optics. We can also use smaller spots (less power/cell) size which might not illuminate the entire cell but may excite enough fluorescence for high SNR imaging. If we could use 20 mW continuous laser energy per target, the net average power for imaging 10-12 neurons of 200-240 mW is lower than previously estimated damaged thresholds for 20 minutes of continuous illumination, which causes ~5°C increase(67). Empirical tests we performed showed that one second of total holographic laser energy even exceeding 1W, when distributed across a large volume, drives only 1-2 degrees of brain heating, which returns to baseline in additional 1 second (**Fig. 6**) (68). Under these conditions, we have probed post-mortem brain tissue for markers of heat-induced toxicity but not observed them(2). Theoretical modeling combined with further empirical measurements similarly estimated that illuminating up to 100 cells with powers similar to those proposed here can be done without exceeding damage thresholds provided illumination isn't continuous but episodic(2). Thus, when attempting to scale voltage imaging to more neurons we can adopt brief imaging trials (0.5-1 second) with sufficient inter-trial intervals to allow for any dissipation of accumulated heat. We will confirm these expectations with micro-thermistors and probe the limits of what powers the cortex can accommodate and over what time periods. We will execute physiological tests of the impact of heating by measuring visual stimulus responses in V1 before and during holographic illumination with various spot numbers. If necessary, we will explore the possibility of actively cooling the imaged area by perfusing a chilled solution over the cranial window. Brain heating will impose an upper bound on how many neurons the system can simultaneously image with 2p excitation. However, optimization of the voltage sensor and the optical system will increase the size of the imageable ensemble.

Brain Motion: Unlike raster-scanning approaches, random access microscopy (RAM) approaches, including holographic excitation, cannot be corrected for post-hoc through image registration. If the brain moves enough on a trial, the cells of interest might move out of the static excitation focus causing artifactual changes in signal. The simplest solution is to exclude trials with significant motion, and in our experience, in well pre-prepared animals only a few trials need to be excluded(2). If there is brain motion, it will be readily apparent as a correlated change in all the concurrently illuminated cells, and thus easily detected and rejected. Alternatively, we could simultaneously raster scan a small portion of the field of view with the galvo-scanning arm of the microscope to detect and reject such trials. Another approach would be to deliberately illuminate each cell with a spot size that is larger than the typical neuronal soma reducing the fraction of motion trials where the cells move out of the excitation focus (at the expense of wasted laser energy). A more technically sophisticated solution would be to track and correct for brain motion in real time with a feedback-controlled galvo-galvo pair.

Aim 2: Develop optimized ASAP4 variants and potent ultra-blue opsins for spectrally separable imaging and optogenetics. 2.1 Develop sensitive and photo-stable red-shifted ASAP sensors. Red-shifting the excitation and emission spectrum of ASAPs will be highly advantageous for three reasons: first, we must be able to use an excitation wavelength for ASAP that minimizes activation of the co-expressed opsin. Second, commercial fiber lasers that are needed to provide high pulse energy for efficient holographic excitation emit at ~1030-1040 nm. Although one can pump an optical parametric amplifier (OPA) with a ~1030 nm laser to provide tunability, these systems are energy inefficient. Third, redder emission light is less absorbed and scattered by brain tissue(69). Importantly, all of our pilot data above were collected with the new GEVI variant ASAP4.4 and using a 1040 nm laser which. Despite only achieving ~35% of maximal excitation of ASAP4.4, this still provided high quality voltage signals. These data demonstrate that ASAP4.4 is sufficient to report action potentials when excited at this wavelength. Although we will continue to develop ASAP4.4 for further red-shifts, we propose to develop ASAPs based on circularly permuted red fluorescent proteins which will allow us to use lower illumination energies. This would increase the number of cells we can image at a time with the same laser energy while also minimizing unwanted excitation of the co-expressed opsin in all-optical experiments).

Several red GEVIs have been engineered to date, although none are expected to perform well with 2p excitation *in vivo*. FlicR1 utilizes CVSD as the sensing domain and a circularly permuted RFP mApple (cpmApple)(70). However, FlicR1 demonstrates very limited sensitivity(47)(71), low brightness, moderate kinetics, and poor performance for *in vivo* study so far. Red GEVIs based on Archaeorhodopsin mutants (25)(23)(38) perform well with high intensity visible light excitation, but do to extremely low quantum yield still appear unsuitable for 2p excitation. A third class that fuses a bright red FP to a voltage-sensing rhodopsin, such as VARNAME(71), exhibits fast kinetics, high brightness, and moderate sensitivity. FRET-based GEVIs, including

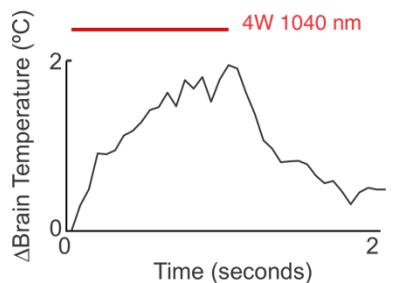


Figure 6: Brain heating by sparse holographic imaging for 1 second of illumination @1040 nm.

VARNAM, perform very well with visible light excitation but have so far not been shown to be suitable for 2p excitation. Therefore, developing a new class of red GEVIs optimized for brightness, sensitivity and kinetics are needed to increase the scale and throughput of 2p voltage imaging.

ASAPs leverage a favorable sensing mechanism and exhibit high brightness sensitivity and fast kinetics, making this design ideal for the next-generation red GEVI. We propose to construct a new red GEVI by swapping the GFP from ASAP with an RFP. The same strategy has been proved successful, which led to many red biosensors using RFPs like mApple (70)(72)(73) mRuby2 and FusionRed(74). We will construct two red versions of ASAP using cpmApple and cpmScarlet and test them first cell culture with imaging and electroporation. Linker optimization is the most effective strategy to improve the dynamic range of ASAP and other circularly permuted FP based biosensors(75)(45). To optimize mApple- and mScarlet-based ASAPs, we will use our established semi-automated high-throughput screening system (**Fig. 7**), which leverages electroporation to measure the dynamic range of each mutant expressed in HEK293-Kir2.1 cells(45). We will use overlap PCR to generate a library with randomization of two positions each time. HEK293-Kir2.1 cells are cultured in 384 microwell plates with conductive glass bottom. The linear PCR products with a promoter can be directly transfected into cells for expression as we demonstrated previously. Electroporation will be applied to change the cell membrane voltage from -70 mV to 0 mV. cpmApple- and cpmScarlet-based ASAP will be screened separately. The red ASAP mutant will be co-expressed with a GFP to compare their relative brightness, which can normalize the variation of expression level. We will also optimize the length of linkers as longer linkers may have complex conformational changes and slow the kinetics. Winners from the high-throughput screening will be further characterized and compared in cultured HEK cells and neurons using patch-clamp electrophysiology. The validated winners will be used as template for the next round of mutagenesis and screening until the products reach satisfactory performance (high level brightness, large dynamic range, and fast kinetics) and outperform previously reported red GEVIs for 2p excitation. Finally, we will characterize new red, soma-targeted ASAP mutants in mouse brain slices and *in vivo* and obtain full excitation spectra in the 2p regime.

Caveats and alternatives approaches: We expect to identify high-performance red ASAP mutants through our high-throughput screening system. While the kinetics of the new indicators might be slightly slower compared to rhodopsin based GEVIs, the sensitivity will outperform most FP-rhodopsin based GEVIs and the brightness should be comparable to that of FP-rhodopsin based GEVIs and much higher than that of rhodopsin based GEVIs. We expect that red ASAPs will be compatible with many optogenetic actuators and green indicators such as GCaMP, and that red ASAPs can be highly excitable with 1030-nm 2p illumination. If cpmApple and cpmScarlet based ASAPs do not perform well as expected, we can swap the cpFP with cpFusionRed orcpmRuby2 which have been used in Ca^{2+} indicators(74)(37). As an alternative strategy, we will also attempt to mutate ASAP4.4 to yellow-shift its excitation spectrum as has been done to convert jGCaMP7 into jYCaMP(76) which makes it far more excitable at 1030 nm.

2.2. Engineer ultra-potent, highly blue-shifted opsins for spectrally separable 2p imaging and optogenetics. To enable all-optical read/write control we must identify or engineer an opsin that is spectrally separable from the voltage sensor and also permits potent control over neural spiking. For ASAP4.4 and future red-shifted ASAPs, we must choose among the bluest opsins yet identified so as to minimize spectral overlap. PsChR2 (77) has the most blue-shifted absorption spectrum, to our knowledge, of all characterized microbial opsins (similar to TsChR, and blue-shifted to Cherrish)(78)(23). It is about ~3x more potent than ChR2 but about 30 nm blue-shifted in its absorbance spectrum while also showing fast kinetics(77). The goal of this sub-aim is two-fold: to blue-shift PsChR2's absorption spectrum further, and thus eliminate any excitation at 1030 nm (**Fig. 8a**), and to increase its overall potency to facilitate optogenetic control. We will build on our prior experience in which we employed structure-guided design to develop extremely potent variants of the opsin Chronos(2)(61), including variants with substantial blue-shifts in their spectra(61).

Pilot data shows that we can drive visual cortical L2/3 pyramidal cells expressing soma-targeted PsChR2 reliably with pulses of excitation light (**Fig. 8b**, top). This demonstrates that the native PsChR2 is sufficient for optogenetic control. More importantly, illumination of neurons co-expressing PsChR2 and ASAP4.4 with 1040

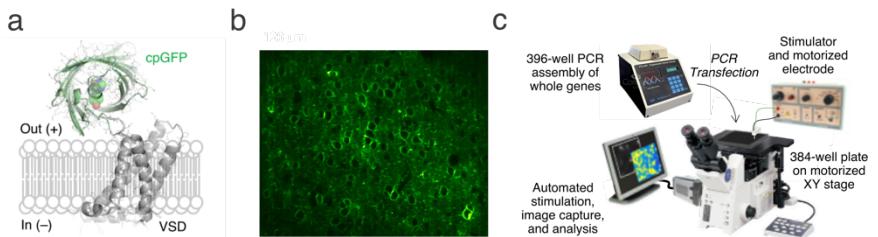


Figure 7: Engineering of ASAP GEVIs. a) Schematic of ASAP structure. b) Example *in vivo* 2p scanning image of L2/3 ASAP-expressing cortical neurons. c) Schematic of the high-throughput screening pipeline for GEVI optimization.

nm light at light levels needed for voltage imaging produced only small depolarizations through the opsin, although they were still detectable (~1-5 mV) (**Fig. 8b**, bottom). This demonstrates that PsChR2 is a promising backbone for mutagenesis to further blue shift its absorbance spectrum and make it more potent. Fortunately, there is ample experimental, structural, and computational detail on how the protein environment of the retinal chromophore of opsins control their spectral properties(77)(78)(79)(80)(81)(82). PsChR2 shares significant sequence homology to many characterized opsins, including ChR2(77), and therefore insight from the large amount of work on ChR2 and related opsins will help guide our efforts. We will first build structural models based on the crystal structures that are available for a number of cation microbial opsins; from these models we will identify the homologous residues that should interact with the chromophore and therefore represent logical first targets for mutagenesis (**Fig. 8c**). We will also leverage our recent success in obtaining electron microscopic (EM) structures of opsins and solve the EM structure of PsChR2. To our knowledge no one has yet reported EM structures of any microbial opsins, and doing so will be of general value for opsin characterization. Obtaining the structure of PsChR2 will provide crucial insight into the molecular determinants of its natively blue-shifted absorption spectrum, identifying the specific residues and amino acid differences compared to related opsins that might have blue-shifted its spectrum. Identifying these specific residues and their orientation in the chromophore binding pocket can help us model and predict the mutations that could further shift its spectrum.

Based on a homology model with other opsins and prior work on PsChR2, we found that a single point mutation from glutamate to aspartate at amino acid 106 blue-shifted its spectrum by 10-15 nm (**Fig. 8d**). This shows that PsChR2_{E106D} is a suitable opsin for spectral separation from ASAP4.4. We will engineer further blue-shifted PsChR2 mutants that also exhibit higher photoconductance and light sensitivity, which is important for scaling up the number of neurons one can photo-stimulate at a time with 2p holographic excitation. We found that a point mutant from phenylalanine to tyrosine at amino acid 209 shows a nearly 3-fold increase in light-evoked currents compared to wild-type PsChR2 (**Fig. 8e**). We will combine this mutation with the foregoing one to attempt generate both blue-shifted and highly potent new variants of PsChR2. These results imply that further mutagenesis should yield mutants with highly improved potency and spectral properties suitable for 2p optogenetics and spectral separation from voltage imaging. Based on the homology model and then based on the EM structure we obtain, we will systematically mutate additional residues that line the ion channel pore to increase its predicted diameter and electrostatic environment to increase ion flux and thus its photo-conductance.

Caveats and alternative approaches: It is possible that we won't be able to sufficiently blue-shift PsChR2 to avoid activation at 1030 nm, although our preliminary data already show that a single point mutation might be sufficient. It is also possible the blue-shifted opsins might be too weak for 2p optogenetics, although the F209Y mutation appear to increase PsChR2 photocurrents several fold. Our prior success enhancing the opsin Chronos by nearly 5-10 fold, which has ample sequence similarity to PsChR2, gives us confidence that this should be possible. Obtaining the atomic structure of PsChR2 (via EM or crystal structure) should further facilitate this process. However, as an alternative we will pursue parallel mutagenesis strategies on other blue opsins including Chroiff and ChroMD, a point mutant of Chronos that is both very light-sensitive and substantially blue-shifted(**Fig. 8f**, (61)).

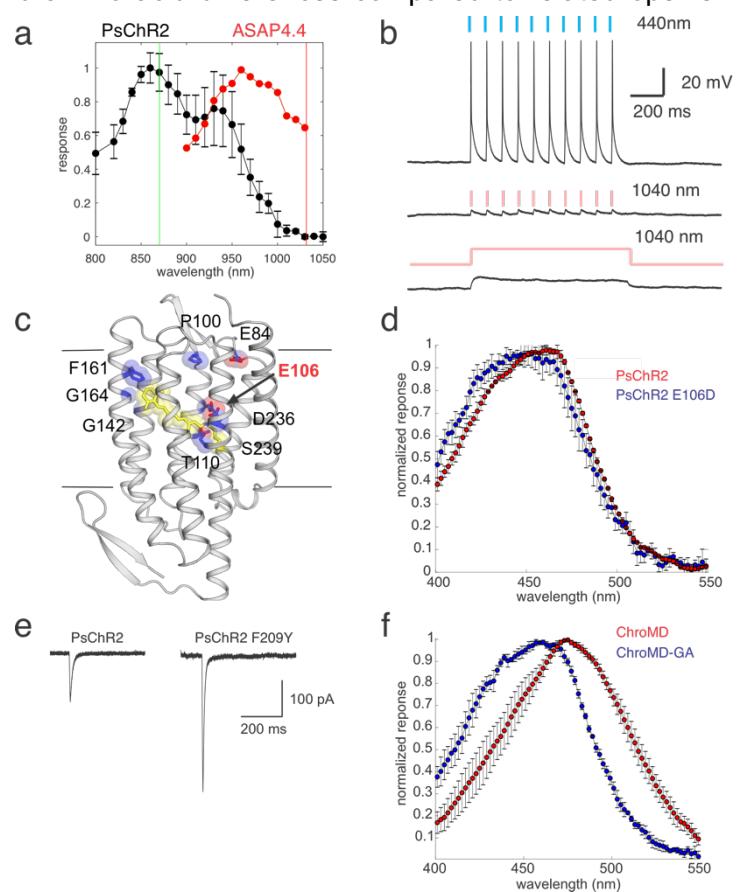


Figure 8: Optimization of a deep blue opsin, PsChR2. a) 2p excitation spectrum of PsChR2 and ASAP4.4. b) Robust optogenetic activation of PsChR2-expressing neurons (top) and minimal activation by pulses (middle) or prolonged (bottom) 1040 nm 2p excitation (bottom). c) 3D homology model of PsChR2 with candidate residues for mutating to blue-shift and increase its potency highlighted. d) Targeted mutagenesis blue-shifts PsChR2 even further. e) A different mutant increases photo-currents. f) Blue-shifting a Chronos-based opsin, ChroMD.

Aim 3: Develop high-throughput all-optical, all-holographic 2p microscopy

Aim 3.1: Build and validate a multi-spectral holographic 2p microscope for simultaneous voltage imaging and 2p optogenetics: To enable simultaneous holographic voltage imaging and holographic optogenetics, we will build a novel, multi-spectral 3D-SHOT microscope with parallel excitation paths for voltage sensors (@1030 nm) and blue opsins (~850 nm). The system has three 2p excitation paths: a conventional raster-scanning path for structural and calcium sensor imaging, and two 3D-SHOT paths, one for voltage imaging and one for optogenetics. For standard imaging we will use a Ti:Sapphire laser (Coherent Chameleon); for the holographic paths we will use a 60W femtosecond fiber laser (Coherent Monaco, 1 MHz) pumping an optical parametric amplifier (Opera F) to provide two beams with high energy output across a broad tuning range (700-1300nm). The system will supply ~5W at 850 nm for optogenetic stimulation and ~10W at 1040 nm for voltage imaging, with an expected net throughput through the microscope objective of 1-2W at each wavelength, which should be sufficient to both image and photo-stimulate 10-20 neurons in a single hologram based on our pilot data. Following our previously established procedures (currently available as a native component of ScanImage software) we will precisely calibrate all three systems in three dimensions so that they are in micron-scale alignment (using excitation of a fluorescent slide and imaging via a substage camera).

To validate the system, we will first measure performance in brain slices with simultaneous electrophysiology. We will prepare mice that co-express ASAP4.4 and PsChR2 in L2/3 pyramidal cells via *in utero electroporation*. We will patch-clamp co-expressing neurons and illuminate them with the voltage imaging path while measuring their fluorescence on an EMCCD camera. We will then simultaneously photo-stimulate these neurons with trains of optogenetic excitation pulses at different pulse frequencies as well as with random ‘Poisson’ trains of stimuli that mimic more physiological activity *in vivo*. We will benchmark both the optogenetics and the voltage imaging data against the electrophysiological recordings. For the optogenetics we will quantify the fraction of successfully evoked action potentials as a function of stimulation frequency and laser power. For voltage imaging, we will quantify the fraction of action potentials successfully detected optically versus the ephys data. Next, we will determine the system’s ability to generate and image sub-threshold potentials by photo-stimulating with optogenetic light pulses of varying intensity. We will assess the system’s detection threshold for postsynaptic potentials (PSPs) of various sizes both on single trials and after trial averaging. Our pilot data above shows that we can easily resolve PSPs of 10-20 mV on single trials. Next, we will execute analogous tests *in vivo*. We will make whole-cell recordings from co-expressing neurons at different depths from the brain surface (100-300 μ m) and repeat these procedures. We will assess how the efficacy of optogenetic stimulation and voltage imaging vary as a function of depth to measure the limits of the system. As we develop new variants of ASAP4 and PsChR2 we will retest neurons expressing these new sensors and actuators with these same procedures to benchmark improvements in performance. Finally, to probe how the system performs when targeting populations of neurons, we will illuminate up to ~16 spots simultaneously, while patching one of these neurons. We will then execute similar tests as above and measure across all the illuminated neurons our ability to resolve light-evoked action potentials and sub-threshold potentials as above.

An all-optical electrophysiology experiment: A typical all-optical electrophysiology experiment will run in several steps: first, we will prepare mice with cranial windows that virally co-express ASAP4.4, PsChR2, and the spectrally separate red calcium sensor jRGECO1a(83) in mouse V1. At the beginning of each experimental session we will identify the location of cells with conventional 2p imaging in 3D via the voltage sensor’s fluorescence. Next, using the co-expressed red calcium indicator we will determine their basic physiological properties. Calcium imaging has low temporal resolution but facilitates densely sampling the activity of each labeled neuron in the volume to identify groups of neurons with specific response properties (such as a common receptive field location or orientation tuning). This will allow us to then choose specific ensembles of neurons to target for simultaneous voltage imaging and optogenetics at much higher speed. We will holographically illuminate 20-40 highly visually responsive neurons and measure their visual response properties with 2p voltage imaging. To assess the accuracy of the voltage imaging approach we will compare the tuning obtained via calcium imaging to that acquired via voltage imaging (**Fig. 9**). To demonstrate its utility, we will measure each neuron’s receptive

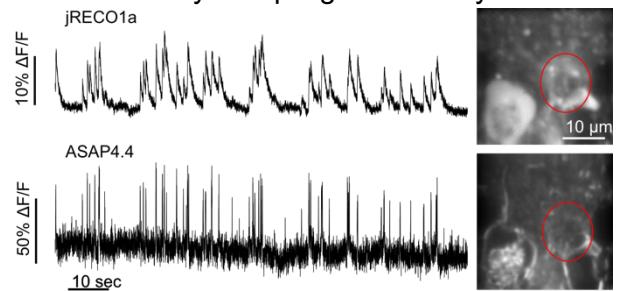


Figure 9: Calcium imaging (top, jRGECO1a) and voltage imaging (bottom, ASAP4.4) from the same neurons.

field with fast reverse correlation(84). To assess fine timescale spike timing we will play natural movies to an awake mouse which drive sparse but reliable temporal patterns of action potentials with millisecond scale dynamics in V1(85)(86)(87). We expect to observe highly time-locked spikes to specific frames in the movie that are reliable across trials. Finally, to demonstrate the power of the system for cell-type specific analysis, we will execute similar experiments on cortical interneurons subtypes, measure their response properties, correlate their spiking with activity of other neurons in the network, and compute pairwise cross-correlograms (88).

Following the imaging paradigm above, in the same mice we will select specific ensembles of neurons for simultaneous imaging and optogenetic activation. First, we will stimulate neurons with fixed pulse trains and image their response. Then, using the natural movie induced activity, **we will attempt to recreate the exact spatiotemporal sequence with millisecond timing** and correlate the holographically evoked pattern with the visually pattern as a metric of success. From this data we will determine the temporal resolution and temporal fidelity of generating and measuring action potentials all-optically.

Caveats and alternative approaches: A primary concern for the all-optical electrophysiology is spectral cross-talk in both directions. Unwanted activation of the opsin by the voltage imaging excitation will directly affect circuit physiology and we must therefore minimize it as much as possible. As described above, we will develop and leverage novel opsins that absorb minimally at >1030 nm, the wavelength at which we will excite ASAP4.4 and the new red-shifted variants we will develop. If that is not sufficient to eliminate cross-talk, we will leverage the more red-shifted voltage sensors we are developing and excite them at 1100-1200 nm, wavelengths that minimally activate both PsChR2 and a host of other potent opsins, such as CoChR and the highly sensitive Chronos mutant ChroMD. The same microscope we will build for exciting ASAP at 1030 nm can also operate at these longer wavelengths. The converse concern is that exciting the opsin will generate optical artifacts in voltage imaging because ASAP and related sensors also absorb at shorter wavelengths. While this type of cross-talk will not impact the physiology of the system, it will corrupt the measurement signals. For brief pulsatile optogenetic stimulation these illumination periods can simply be excluded from analysis. However, we will also pursue a strategy in which we optically delay the femtosecond pulses for imaging relative to those for optogenetics (the pulses are initially synchronous as they are emitted from the same laser system) and then trigger image acquisition after each optogenetic pulse to avoid its contaminating effect. Another concern is brain heating, which will place constraints on how many neurons we can image and photo-stimulate at a time. Since holographic imaging will often require continuous illumination, while optogenetic stimulation only requires pulsatile illumination, most of the heating will be from voltage imaging, which is addressed above.

Aim 3.2: Build and integrate ultra-fast (10 kHz) MEMS-SLMs for both imaging and optogenetics to substantially increase speed and throughput of the system. Although commercial high pixel count SLMs can generate complex holograms to illuminate dozens to hundreds of cells at a time (see **Fig. 3**), the more spots in the hologram, the lower its effective ‘contrast’(21) which will lower the precision of optogenetic stimulation. More illumination spots will also lead to greater mixing of voltage sensor emission on the camera due to scatter. Both of these problems can be mitigated by maintaining high sparsity in each hologram and ensuring that the illuminated targets are relatively far apart in the FOV. However, this spatial sparsity constraint will limit throughput and constrain the possible geometries of imaging and photo-stimulation. To address this problem, we could employ holograms that only illuminate a small number of sparsely distributed cells and then rapidly interleave phase masks on the SLM. Conventional SLMs, however, can refresh only at 300-500Hz, which is far too slow to attempt to interleave holograms for kHz voltage imaging and optogenetic stimulation. Thus, we need SLMs that can refresh at least at 2kHz to obtain any increase in throughput. Novel MEMS-SLMs that can refresh at up to 5-10 kHz speed break this barrier for the first time. These systems will allow us to temporally interleave up to 10 3D-holograms per millisecond for a 10-fold increase in the size of the imaged and photo-stimulated ensemble.

The Muller lab has previously developed a modular array-format design approach to micromirror-based optical tools that combines a semi-custom commercial fabrication process (MEMSCAP’s PolyMUMPs + MUMPs-Plus service) with a comprehensive simulation pipeline that can evaluate the impact of pixel-to-pixel variations via Monte Carlo-based methods(89). As a demonstration of this platform, we produced and successfully operated a concentrically-wired micromirror array for random-access, dwelling-capable focus tuning at low voltages (<30 V), across wavelengths of up to 1,100 nm, and at a refresh rate >15 kHz (**Fig. 10**)(90). We also developed an application-specific integrated circuit (ASIC) capable of providing 6-bit analog drive at a refresh rate of up to 10 kHz and across an array of up to 200x200 drive pixels that can be coupled to a MEMS micromirror array (**Fig. 10d**)(27). In order to achieve high-density integration with pixel-level addressability between our MEMS micromirror arrays and ASIC drivers, we devised an expanded version of our existing fabrication process with the addition of a routing layer directly underneath the micromirrors (**Fig. 10f**). This layer will serve to route

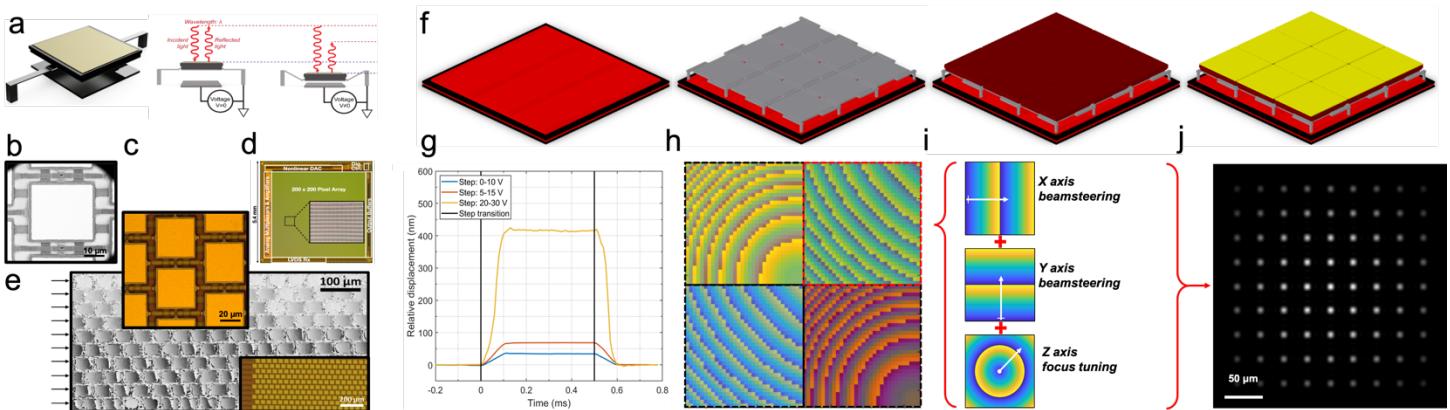


Figure 10: Design, fabrication and operation of a MEMS-SLM. a) 3D rendering and principle of operation of electrostatic piston-motion micromirrors as analog phase-shifting pixels. b) Scanning electron micrograph of fabricated pixel. c) Optical micrograph of fabricated array. d) Optical micrograph of developed driver ASIC. e) Phase reconstruction image of a sample array under actuation. f) Expanded fabrication process for the development of high-fill factor micromirror arrays capable of pixel-level addressing. g) Experimentally measured settling response (>5 kHz) of current micromirror prototypes. h) Example phase mask produced by 64x64 micromirror array for 4-point targeting. i) Each 32x32 sub-block is responsible for 3D scanning of a single point. j) Stitched image grid of simulated scanning performance for single 32x32 array sub-block across one quadrant of the total field of view.

traces to the periphery of the arrays for a board-level integration approach that can accommodate array sizes of up to 128x128 via fanout and wire-bonding. Under this integration scheme, the ASIC driver would be patterned with solder bumps for direct ball grid array integration to the same printed circuit board that will house the micromirror array. The MEMS micromirrors will have a similar stack-up to that of our first-generation structures, with the driving electrodes being deposited below the grounded and suspended mirror structures. The updated micromirror structures will also incorporate improvements to shore up performance robustness and uniformity for optimal diffraction efficiency, including the use of 4 suspension beams per mirror instead of 2, a planarization step that will ensure flat mirror surfaces, and an increase in pixel fill factor from 70% to 94% (Fig. 10f).

We verified in simulation that high-speed multi-point scanning can be achieved at array formats allowable under this fabrication scheme by partitioning arrays into sub-blocks that would be independently responsible for the 3D translation of distinct points (Fig. 10h-j). At a system level, four separate arrays could be optically tiled to address four non-overlapping quadrants covering the total target field of view for temporally multiplexed readout. In addition, such an optical patterning approach benefits from direct phase mask calculations that reduce computation time by roughly two orders of magnitude compared to the conventional iterative phase retrieval methods used in CGH (e.g., Gerchberg-Saxton). We have also identified a path for the integration of large-format SLMs (with array sizes on the order of 1000x1000) that builds upon this same fabrication process by preprocessing our substrate MEMS wafers with polysilicon-based through-silicon vias (TSVs) to provide each micromirror pixel with a front-to-backside electrical connection. Following micromirror processing, direct chip-to-chip thermocompression bonding between the backside of the MEMS and the frontside of the ASIC drivers would enable a compact and scalable integration approach that would allow us to dramatically raise throughput and address a larger FOV.

Although these initial MEMS-SLMs have fewer pixels than conventional LCoS-SLMs, they are ideally suited for generating a small number of fixed-sized illumination spots and steering each spot independently at very high speed in 3D. Thus, these systems make a tradeoff well-suited to our task: they sacrifice the ability to make highly complicated 3D holograms at slow speed, for the ability to precisely generate and steer a small number of cell-sized illumination spots at very high speed (~ 16 spots/ $100\ \mu s$). We will integrate a MEMS-SLM into our dual-holographic illumination microscope, one for voltage imaging and one for optogenetics. A schematic of the initial microscope design is in Fig. 11. Following the procedures above, we will align and calibrate the system, then validate it with simultaneous voltage imaging, optogenetics, and patch-clamp electrophysiology in brain slices and *in vivo*. We will systematically test the effective spatial and temporal resolution of the new system, acquiring optical and ‘physiological’ point spread functions that measure the spatial fidelity of the system with whole-cell recording both with single spot illumination and when projecting up to 16 spots in both the imaging and optogenetic excitation paths. We ultimately aim for an addressable volume $500\times 500\times 200\mu m$ using appropriate demagnification and multiplexed SLMs (seem Aim 3.3, below). To capture the fluorescence emission generated by each illumination wavefront we must employ an imaging system that can operate at the same speed. Initially, we will use the same high speed EMCCD camera as above with fewer read lines, although higher

performance systems with 4x the pixels and sampling at 10 kHz sampling rate could be used (Nüvü Hnū 240). By synchronizing the SLM refresh with triggering of EMCCD frame acquisition, each image (~0.1-0.2 ms exposure) will capture a different illuminated group of neurons, up to 160 unique neurons per ms.

Caveats: The main limitation for this approach is whether there is sufficient signal in such short exposures. This can be partially compensated for by increasing laser excitation intensity, although this is bounded by tissue heating or damage. Brighter, red-shifted GEVI variants with peak absorption at ~1030 nm will mitigate these concerns, though we expect there to be a tradeoff between throughput and the SNR of the system. Depending on the goals of a specific experiment, one can strike the appropriate balance between these tradeoffs.

Aim 3.3: Pilot spatiotemporal multiplexing of holographic paths to increase system throughput. To increase the number of neurons the system could simultaneously image and optogenetically control, as a proof-of-concept we will temporally multiplex multiple femtosecond SLM illumination paths. Prior studies have increased the throughput of laser scanning imaging by splitting and delaying the laser into multiple imaging beams that are focused to different points in the tissue(91)(92)(93)(94)(95)(96)(57). We will adopt an analogous approach but for holographic illumination(6). Multiplexing multiple SLMs at the nanosecond timescale will allow us to illuminate many more neurons while maintaining the same sparsity of each illuminating wavefront. The high-energy femtosecond laser operates at 1 MHz, which provides 1 μ s between laser pulses, ample time to delay multiple multiplexed beams. Each parallel beam path will illuminate its own SLM. We will thus split both the voltage imaging and optogenetic paths into two parallel multiplexed beams, each projected onto their own 10 kHz SLM. A single split to two SLMs will double our intended scale from 160 to 320 neurons at 1 kHz. To acquire fluorescence readout, we will employ multi-anode PMT arrays (16x16 detector) with fast parallel readout electronics (Vertilon Corp).

The limits of this multiplexing are the available laser energy, the amount of laser energy the brain can accommodate, and the requirements for aligning the various light paths. The major advantage is that each additional pathway linearly increases the size of the optically addressable ensemble without any compromise in the spatial fidelity of each individual wavefront or loss of signal. Furthermore, the GEVI emission driven by each multiplexed phase mask can be sampled entirely independently from the PMT array. To achieve this, we will pilot the use of high speed multianode PMT arrays (Hamamatsu) with custom designed readout electronics operating at >4MHz (1 read/femtosecond pulse).

Conclusion: We aim to develop and rigorously validate a new form of electrode-free all-optical all-holographic electrophysiology. It capitalizes on important advances in 2p CGH and 2p excitable voltage sensors and optogenetic proteins that we and others have made. This system will be able to measure and manipulate neural activity with cellular resolution and millisecond precision across a large volume of neural tissue simultaneously *in vivo*. Ultimately, by developing even brighter, red-shifted GEVIs, ultra-potent blue-shifted opsins, larger format MEMS-SLMs together with multiplexed illumination paths and optimized sensing algorithms, it is possible that future advances could permit sampling and control of the voltage of up to 1,000 neurons at kHz speeds. Such a system could lead to transformative advances in our understanding of brain function, plasticity and disease.

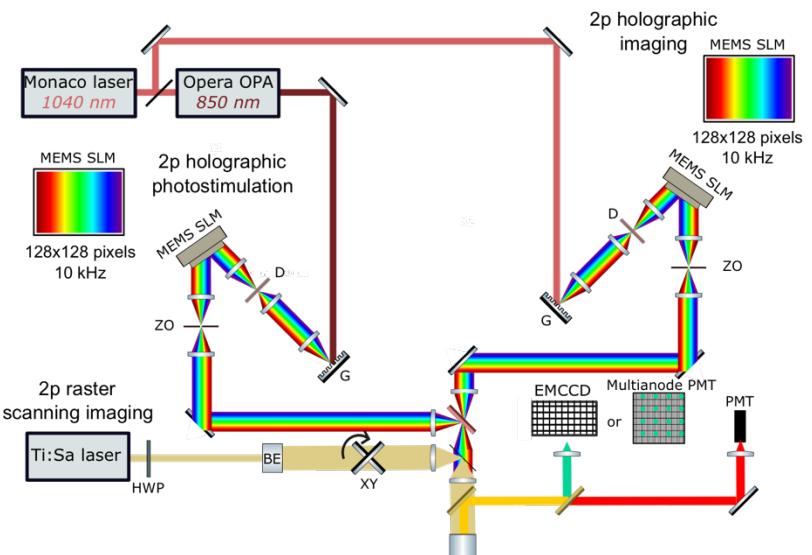


Figure 11: Full optical schematic of the multispectral holographic microscope.

Approximate Timeline

	Year 1	Year 2	Year 3
Aim 1	1.1 Validate of 2p voltage imaging 1.2 develop initial holographic imaging scope 1.3 Develop imaging algorithms 1.4 Benchmark against FACED		
Aim 2		2.1 Develop and optimize red ASAP GEVIs 2.2 Develop and optimize blue-shifted opins	
Aim 3		3.1 Build multi-spectral scope 3.2 Build and integrate MEMS-SLMS	3.4 Build and test multiplexed system

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

Yes No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved

Yes No

Is the Project Exempt from Federal regulations?

Yes No

Exemption Number

1 2 3 4 5 6 7 8

Other Requested Information

VERTEBRATE ANIMALS

RODENTS

All animal work will be done by Dr. Adesnik's laboratory in Life Science Addition animal facility managed by UC Berkeley's Office of Laboratory Animal Care. The facilities are managed and maintained by full-time veterinarians. We will need approximately 75 mice per year between the Adesnik and Jilabs. All animals will be bred in our facility. The mouse is an excellent experimental model for the study of neural circuits and has been used in numerous published studies of cortical function. Both female and male mice will be used for experiments in equal numbers.

1. Description of Procedures

- a. **Maintenance of mice:** Breeding animals are housed in a state of the art animal facility that is fully staffed. Breeding animals are housed three per cage (two females and one male) at most. Pups are weaned at three weeks, identified by ear tag and genotyped by clipping the end of the tail.
- b. **Tissue harvesting for acute brain slices:** Animals (P21-P28) will be deeply anesthetized by isoflurane inhalation with saturated vapor in a bell jar. Following complete anesthesia animals will be rapidly sacrificed by decapitation with sharpened scissors and their brain removed for sectioning.
- c. **In utero electroporation:** Pregnant female mice (E15) will be anesthetized with isoflurane (1.5-2.5%), the abdomen cleaned with sterile alcohol swabs and then betadiene, and sterile instruments used to expose the uterine horns. Plasmid DNA will be introduced to the lateral ventricle of each embryo with a sterilized glass needle via pressure injection, and electric shocks (40 V, 1s) will be applied to each embryo via electrodes. The uterine horns will returned to the abdomen, the peritoneum and skin sutured closed, and sterilized with alcohol and betadiene. Buprenorphine will be given as analgesic and the animals monitored for full recovery at 12 hour intervals.
- d. **Preparation for imaging:** Mice (ages P28-65) will be anesthetized with isoflurane (1.5-2.5%) and implanted with a titanium head post that will be secured in place with dental acrylic. A small craniotomy will be made above the primary sensory or prefrontal cortex for both optical and physiological access. The craniotomy will be protected with a glass coverslip, sometimes with a small hole for electrode access. For non-electroporated mice a small volume (50 nanoliters) of adeno-associated viruses driving a fluorescent calcium indicator will be slowly injected into the barrel cortex, visual cortex or the prefrontal cortex. For mice to be imaged with micro-endoscopy, a GRIN lens (0.5 mm diameter) will be implanted over the prefrontal cortex and sealed with dental cement. Animals will be given buprenorphine as an analgesic and monitored for full recovery following surgery at 12 hour intervals. Several days after recovery animals will be slowly habituated to head restraint in a custom system that allows free running on a circular treadmill. Over several periods of restraint, sessions will be increased up to 1-1.5 hours. Animals that do not habituate (although extremely rare) will be removed from the study.
- e. **Imaging in sedated, anesthetized mice:** Mice will be anesthetized with isoflurane (0.5-1%) and sedated with chlorpithixene (5 mg/kg) and fixed in a frame under the microscope.
- f. **Imaging in awake, head-fixed mice:** Animals will be restrained by head-fixation under the microscope. Imaging sessions will last 1-2 hours. All experiments will be conducted in a dark, sound attenuated chamber monitored with an infrared video camera system.

2. Justification

Understanding sensory function of the cerebral cortex requires research on animals. Mice are the most suitable mammalian species for genetic studies because of their short generation time and manipulability of the germline.

3. Minimization of Pain and Distress

No distress or discomfort is caused to animals being euthanized. Isoflurane gas anesthesia is used for surgical procedures such as stereotaxic survival surgery experiments. Buprenorphine and carprofen are

provided to minimize post-operative pain. Procedures followed are standard in the field, and are IACUC-approved. Any animals showing undue signs of pain or distress are immediately euthanized to minimize suffering.

4. Method of Euthanasia

All methods are consistent with of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals. Following collection of sufficient data from each animal, animals will be euthanized for tissue recovery and histological verification of recording site. Mice will be deeply anesthetized with ketamine and xylazine and perfused transcardially. Following complete cardiac failure the brain will be removed. The euthanasia method specified is consistent with the recommendations of the Panel of Euthanasia of the American Veterinary Medical Association.

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CONSORTIUM / CONTRACTURAL AGREEMENT

The University of California, Berkeley will engage with Stanford University. Dr. Hillel Adesnik and Dr. Michael Z. Lin will act as the PIs for their respective sites. They will coordinate the day-to-day research conducted at their institutions, as well as work to set and revise overall project goals, assist with experimental design and analysis, and co-mentor postdoctoral and graduate student trainees from all sites. All sites' administrative staffs are highly experienced in handling NIH consortium subcontracts.



Friday, April 23, 2021

Prof. Rikky Muller
S. Shankar Sastry Assistant Professor in Emerging Technologies
Department of Electrical Engineering and Computer Sciences
University of California, Berkeley

Dear Rikky,

I am very happy to support your NIH R01 proposal entitled, "All-optical all-holographic two-photon electrophysiology". The kHz-rate holographic optical read/write capability would be a major advance in the throughput of ensemble-level functional neural mapping.

At Boulder Nonlinear Systems, we have been developing holographic photostimulation tools based on liquid crystal on silicon (LCOS) devices for many decades. From the beginning, our neuroscience collaborators and customers have pushed us in the pursuit for higher speeds, resulting in our development of high-resolution LCOS modulators exceeding 500 Hz frame rate (Marshel *et al.*, *Science*, **365**, 6453, 2019). However, we are beginning to push the limits of what can be achieved with liquid crystal, and your MEMS architectures provide the most promising means of breaking the kHz barrier.

Through the development of the parallel, multispectral, read/write optical paths, the high-speed GEVIs and opsins, and the protocols to utilize these developments together effectively, the proposed research would be greatly beneficial to realizing the full potential of our ongoing collaboration to increase the MEMS resolution and bring this promising technology to market (NIMH STTR 1R41MH125556).

I am very excited for you to develop this novel microscope architecture and the protocols that will leverage this capability. I fully support this effort and look forward to continuing our collaborations to advance this field.

Sincerely,

A handwritten signature in black ink, appearing to read "Christopher Hoy".

Christopher Hoy, Ph.D.
Vice President, Business Development & Technology Integration
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Hillel Adesnik, PhD.
Associate Professor of Neurobiology
New York Stem Cell Foundation Robertson Investigator
Department of Molecular and Cell Biology and HWNI
University of California, Berkeley

Dear Mr. Adesnik,

I am pleased to provide you with this letter detailing how Vertilon would support your research project for two-photon excitation voltage imaging. This is a very exciting opportunity for my company as I believe that Vertilon would be quite successful in meeting your project goals, and that the development of this technology could potentially lead to more new product possibilities for Vertilon.

As you know, Vertilon is a small, privately-owned company that specializes in instrumentation for scientific research. Our standard product line includes equipment specifically used for high-speed, multichannel data acquisition from silicon photomultipliers, multianode photomultiplier tubes (MAPMTs), and photodiode arrays. As a small company we have been able to intensely focus our resources on the markets for these products and as a result have become the leading supplier of this highly specialized instrumentation. Vertilon's products have been sold throughout the world to universities, government laboratories, and corporate R&D groups for use in diverse applications such as x-ray detection systems, high energy particle physics, muon tomography, flow cytometry, fluorescence detection, PET & SPECT medical imaging, and confocal microscopy. Our products have been used in leading edge research, mentioned in many scientific journals, and are presently being designed into OEM commercial products.

Regarding your project, Vertilon would support you and your team by customizing our standard electronics and software products for sampling of a 256-channel PMT array at MHz speeds. We would propose to design, build, and test electronics and software that would interface to a Hamamatsu 256 anode MAPMT, perform pixel-by-pixel sampling, and transfer this data to a computer for further processing. These custom-designed electronics would be implemented in the form of printed circuit boards containing high speed analog hardware, FPGA digital signal processing functions, and firmware-based algorithms. We would deliver the data acquisition unit as a compact solution packaged in a rugged laboratory type enclosure.

Because of the uniqueness of this project along with our extensive experience with high speed multichannel data acquisition from PMTs, I believe that Vertilon is in a strong position to successfully provide you with a solution. Hopefully, this letter will assist you in your proposal and the successful funding of your project.

Sincerely,

A handwritten signature in black ink that reads "Vincent Palermo".

Vincent Palermo
President

April 29, 2021

RESOURCE SHARING PLAN

This proposal will generate two types of resources that will be shared freely and publicly. The lead PI will oversee all aspects of management of resource sharing and dissemination. First, this project will generate novel opsin and voltage sensors, as well adeno-associated vectors that will be broadly useful for all types of optogenetics and voltage imaging. All DNA plasmids generated under this grant will be deposited at the publicly accessible resource Addgene, including the entire sequence of each DNA plasmid. Plasmids will be deposited that can drive constitutive or Cre-dependent expression of the new opsins and sensors in mammalian cells, and for convenient sub-cloning into other vectors. Plasmids will be suitable either for transfection and electroporation, or for the generation of adeno-associated viral vectors. All validated plasmids will be deposited by the end of year two, and any additional constructs generated in year three will be deposited at the end of year 3. The second resource will be a full optical schematic and parts lists for the construction of the multiphoton digital holographic system described in this proposal. All parts (including catalog numbers) will be listed in a publicly available spreadsheet. The design of the system will be cataloged in full detail, including a complete optical schematic that can be used for the construction and alignment of the optical system. A full description of how the system is to be aligned for proper function will also be published. All documents will be accessible via a public repository, GitHub, by the end of year two, and updated as needed by the end of year 3. The third set of resources will be software, written in the Matlab environment, for the control and operation of the multiphoton holographic microscope. The software will be designed to seamlessly integrate with the open source ScanImage software package, for the operation of conventional laser-scanning two photon microscopes. We have previously contracted with the developers of ScanImage (Vidrio technologies) to develop a software package for 3D-SHOT, the holographic system we previously developed. We will further contract with them for this proposal to extend this software package to this new all-optical system. The software will provide the ability to generate custom holograms off user selected points in a volumetric image (3D stack) captured in ScanImage. The software also includes a full suite for 3D spatial calibration to ensure the two multiphoton paths (scanning and digital holography) are in near-perfect alignment. Software will be extensively documented and routinely updated to correct for any bugs and to implement improved versions of the software.

To transfer this technology more broadly to end users that have limited optical expertise we plan to work with several commercial vendor (Bruker, Scientifica, Neurolabware, and Intelligent Imaging Innovations -3i) to adapt their commercial multiphoton systems for the optimized holographic system developed in this project.

In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. Material transfers will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. Berkeley will adhere to the NIH Grant Policy on Sharing of Unique Research Resources including the "Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts" issued in December, 1999.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

DNA plasmids will be generated by this proposal. For validation, all plasmids will be fully sequenced, transfected into cultured cells, and assay electrophysiologically for proper function. Fully validated plasmids will then be deposited into Addgene. For pAAV plasmids for viral construction, plasmids will further be used for production of AAV by viral vectors cores, and the resulting viral preparations will be tested by *in vivo* injection into mouse brain, and subsequently assayed electrophysiologically for proper function. Only plasmids that produce functional AAV vectors will be deposited into Addgene, and resulting AAV preparations at external vector cores offered for purchase to end users.