OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED | **Application Identifier**

5. APPLICANT INFORMATION **Organizational DUNS*:** 0468381840000

Legal Name*: OPEN SOURCE INSTRUMENTS, INC.
Department:
Division:
Street1*: OPEN SOURCE INSTRUMENTS, INC.
Street2: 130 MOUNT AUBURN ST
City*: WATERTOWN
County: Massachusetts
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02472-3932

Person to be contacted on matters involving this application
Prefix: First Name*: Kirsten Middle Name: Last Name*: Hashemi Suffix:
Position/Title:
Street1*: 27 Loring St
Street2:
City*: Belmont
County: Massachusetts
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02478-4714
Phone Number*: 16177331553 Fax Number: Email: kirsten@hashemifamily.com

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 141911312

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No 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*
An optogenetic brain implant with EEG monitoring and response for mice

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2019 03/31/2020

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MA-005

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Mr. First Name*: Michael Middle Name: London Last Name*: Collins Suffix:
Position/Title: Engineer
Organization Name*: OPEN SOURCE INSTRUMENTS
Department:
Division:
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: WATERTOWN
County: MASSACHUSETTS
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02472-3932
Phone Number*: 978-335-3137 Fax Number: Email*: collins@opensourceinstruments.com

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$244,433.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$244,433.00
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*: Kirsten Middle Name: Last Name*: Hashemi Suffix:
Position/Title*: Operations Manager
Organization Name*: Open Source Instruments, Inc.
Department:
Division:
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: Watertown
County: MASSACHUSETTS
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02472-3932
Phone Number*: 6177331553 Fax Number: Email*: kirsten@opensourceinstruments.com

Signature of Authorized Representative*
Kirsten Hashemi

Date Signed*
09/05/2018

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

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: OPEN SOURCE INSTRUMENTS
Duns Number: 0468381840000
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: WATERTOWN
County: MASSACHUSETTS
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 02472-3932
Project/Performance Site Congressional District*: MA-005

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: Cornell University
DUNS Number: 8726124450000
Street1*: School of Biomedical Engineering, 237 Tower Road
Street2: B57 Weill Hall
City*: Ithaca
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 14853-7202
Project/Performance Site Congressional District*: NY-023

Additional Location(s) File Name:

Funding Opportunity Number: PA-18-871. Received Date: 2018-09-05T11:19:48.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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 ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If 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: 04-13-2018

Animal Welfare Assurance Number A3347-01

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 environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

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*	Project-Summary-Abstract-20180905-0948.pdf
8. Project Narrative*	Narrative-20180905-0946.pdf
9. Bibliography & References Cited	ReferencesCited-20180905-0933.pdf
10.Facilities & Other Resources	Facilities_OSI_Cornell.pdf
11.Equipment	Equipment_OSI_Cornell.pdf
12. Other Attachments	SBC_000814955_(1).pdf 88004-Schaffer-Prop-P1-cover_letter.pdf

Funding Opportunity Number: PA-18-871. Received Date: 2018-09-05T11:19:48.000-04:00

Abstract: Optogenetics can be used to selectively stimulate or suppress the firing of genetically targeted and spatially targeted mammalian neurons. It is used to study neuropsychiatric diseases *in vivo* with mouse models of conditions including epilepsy, schizophrenia, and Parkinson's. Optogenetics may be used as functional neurosurgical intervention for correcting disease states in the brain. It has been previously shown that seizures have the ability to be halted or reduced by optogenetic activation of inhibitory neurons with the use of Channelrhodopsin-2. It has also been shown that expressing Halorhodopsin (HR) in cortical pyramidal neurons can also reduce seizure propagation. By monitoring EEG data in real time, seizures can be identified at their onset and correcting pulses of optogenetic stimulation may be applied. This line of research is currently limited by the lack of suitable instruments. This project proposes the development of a **fully implantable, wireless EEG monitor capable of autonomously detecting EEG events in real-time and applying correcting pulses of closed-loop optogenetic stimulation**. The proposed instrument will be compatible with mouse biology, thus permitting chronic experiments in the enormous pool of transgenic mouse strains available with photosensitive proteins and validated as models of human disease. Aim 1 will develop the necessary hardware by combining core technologies demonstrated in existing products. Aim 2 will enable autonomous EEG event detection in the instrument's micropower logic chip by adapting a computationally efficient algorithm that has been proven capable of classifying EEG events including normal activity, seizures, ictal spikes, inter-ictal spikes, and polyspikes. Aim 3 will test the device's ability to detect seizures, apply correcting optogenetic stimulation, and reduce the duration of focal seizures induced in mice by the nanoinjection of iron chloride. Phase I of this project will make the mouse-compatible instrument available for sale to researchers studying circuit theory of the brain and diseases/disorders characterized by aberrant EEG states such as epilepsy, schizophrenia, Alzheimer's, and obsessive compulsive disorder. Potential follow-on Phase II would develop the technology into a medical instrument that aborts focal seizures in humans who suffer from pharmaceutical-resistant partial epilepsy (approximately 15 million people).

A wireless instrument capable of simultaneous EEG recording and optogenetic stimulation in mice will enable a new class of experiments of epilepsy, schizophrenia, Alzheimer's, obsessive compulsive disorder, and other conditions characterized by aberrant electrophysiology. In addition to aiding basic research and the identification of pharmacological treatments, the instrument will enable a new class of functional neurosurgical intervention. The instrument will demonstrate a new treatment of focal seizures in patients suffering from pharmaceutical-resistant partial epilepsy.

Facilities

Open Source Instruments

Open Source Instruments has its laboratory and manufacturing facility at 5 Pratt Ave, Waltham, MA. Our 2,000 square foot, rented space has an open floor plan. It is well lit with natural and artificial light. There are eight distinct work stations in the space, which are comprised of a work-bench surface, seating, magnifier lights, computers, and specialized equipment. We have three general-purpose electronic assembly stations and an additional three stations dedicated to the manufacture of subcutaneous transmitters. Other stations include an optical fiber stretcher and radio frequency testing space with Faraday enclosure.

The facility is large enough to include space for storing items related to manufacturing such as electronic components, flux, potting epoxy, manufactured parts ready for sale, and Faraday enclosures. There are also shelves for disposable items like mixing tips, paper containers, and wipes. The space is large enough to absorb growth in both projects and people.

The Open Source Instruments Inc. billing and correspondence address is 130 Mt. Auburn Street, Watertown, MA 02472. This is the address under which our paperwork is filed and it is where we receive our mail. Accounting, invoicing, and bill paying happen from this address.

Open Source Instruments is a small company with four regular employees, three of whom are on-site at the manufacturing facility. The relationship between them is collegial. There are several projects on-going within the facility, some of which all participate in and some of which are completed independently. The engineers tend to discuss and trouble shoot each-others' issues. Kevan Hashemi initially recruited Michael Collins, having maintained their relationship while Mr. Collins fulfilled his obligations to the US Services for his education, in order to work closely with him. Mr. Hashemi and Open Source Instruments have supported Mr. Collin's goals and projects and will continue to do so as best we know how, in order to work with a talented, remarkable individual.

Open Source Instruments does not work with any animals in its offices. We have, instead, collaborated with researchers who themselves maintain animal facilities in order to develop all of our implantable devices. We have worked with the Institute for Neurology at University College London, the Department of Physiology at Edinburgh University, Children's Hospital Boston at Harvard University, and Oxford University, for example. For the initial optogenetic device created for a rat, we collaborated with the Institute of Neurology, University College London. For this proposal, we have been fortunate to catch the interest of Dr. Chris Schaffer in the Department of Biomedical Engineering, Cornell University. All animal testing will be conducted under his direction.

Cornell University

Cornell University Department of Biomedical Engineering and the Schaffer Laboratory meet all United State regulations for the ethical treatment of laboratory animals, and their facilities are maintained according to the highest standards. Profs. Schaffer and Nishimura have a co-administered, collaborative lab with shared research space, equipment, and other resources.

Laboratory: The shared laboratory, located at Weill Hall at Cornell University, consists of about 3000 sq. ft. of work space, including areas for animal surgery, wet chemistry, cell culture, mechanical and electronic fabrication, and data analysis. The optical imaging experiments are conducted in two dedicated 800 sq. ft. laser labs, which each house a 140 sq. ft. vibration-isolated optical table on which all laser systems and microscopes are constructed. Two ~100 sq. ft. rooms in the animal facilitate are dedicated to behavioral testing and long-term electrophysiological recording for our laboratory.

Animal: Animal husbandry and housing facilities for rodents are located at Weill Hall. The laboratory includes a fully-equipped rodent microsurgery room with gas anesthesia, physiology monitoring, stereotaxic apparatus,

stereoscopes, and perfusion setup. Cornell University is fully accredited by AAALAC, and provides 24 hour/7 day veterinary care for all research animals, as well as hands-on training in animal procedures.

Cell Culture: A BSL2 facility for cell culture and viral vector development is located in the lab. The space includes incubators for mammalian cells and bacteria, liquid nitrogen storage, a BSL2 laminar flow hood, and a chemical fume hood.

Computer: Extensive dedicated, networked computer systems are available in the laboratory, including a central 172 TB RAID6 data storage system, machines for data acquisition and instrument control (6 workstations), for data analysis and simulation (12 workstations with parallel computing capability plus six laptop computers), and for preparing papers and presentations for dissemination of our data. Data acquisition, analysis software, and simulation code is largely written in house.

Office: PIs have private offices, while lab members share communal office space and a dedicated conference room. Administrative support is provided by the Department of Biomedical Engineering.

Other Resources: Library resources at Cornell are extensive. Online subscriptions to relevant clinical and research journals are available. Professionally-staffed machine and electronics fabrication shops are available at Cornell, as well as student machine and electronics fabrication shops (where students can work after completing required training). A professionally-staffed histological service is available through the Cornell Veterinary College. This group is not only able to help with slicing tissue and standard histology staining, but also with antibody staining.

Research Environment:
These experiments take advantage of unique resources and capabilities available in Prof. Nishimura and Schaffer's laboratories at Cornell University. Few labs have both the resources and expertise in optical design and nonlinear optics as well as in vivo animal work and biology to conduct the proposed studies. The lab has a long history of studying cellular interactions in the normal and disease state central nervous system of rodents using nonlinear optical tools as well as developing novel applications for laser technologies. In addition, Cornell University is a renowned center of expertise for in vivo microscopy and the use of nonlinear optical techniques for biology research. The Schaffer/Nishimura lab both contributes to this technology development and benefits from it, for example in the use of longer wavelength light for very deep in vivo two- and three-photon imaging (in collaboration with Prof. Xu (Applied Physics)). Our lab also collaborates extensively with neurobiologists at Cornell (e.g. several papers with Prof Fetcho (Neurobiology)) and with researchers at Weill Cornell Medical (e.g. several papers with Prof. Iadecola (Neurology) and Prof. Schwartz (Neurosurgery)).

Equipment

Open Source Instruments

In its laboratory, Open Source Instruments has all equipment necessary for electronic design and assembly. Items include:

Six soldering irons at the electronic assembly stations

Three complete telemetry set-ups for testing and programming transmitters, which have LWDAQ Drivers and Octal Data Receivers, antennas, and spectrometers

Optical fiber stretcher to heat and divide optical fibers to create tapers

Two oscilloscopes

One Vector Voltmeter

Photometers

Lab oven for elevating temperature during accelerated testing

Inspection optics

Anti-static mats

Cleaning station with hot water and specialized brushes for electronics

Compressed nitrogen

Compressed air

Vacuum chamber for device encapsulation

Motorized rotators for encapsulation curing

A custom silicone curing enclosure

Custom-made Faraday enclosures

Cornell University

The Schaffer lab comes with the support of the Biomedical Engineering College at Cornell University.

Surgical facility (located in Nishimura and Schaffer laboratories):
Three surgical stereotaxic setups, three high-quality surgical stereoscopes, gas or injectable anesthesia, animal ventilator, blood pressure monitor, two pulse oximeters, exhaled carbon dioxide monitor, five closed-loop core temperature thermometer and heating blankets, animal perfusion setup, micropipette puller. Full histology capabilities, including cryostat, setup for stains as well as immunohistology, and Zeiss widefield fluorescence microscope.

Optical imaging facility (located in Nishimura and Schaffer laboratory):
High-power commercial femtosecond laser oscillators:

1. Manually-tuned, high-power Ti:Sapphire laser: 720-980-nm wavelength, 80-fs pulse duration, 4-W average power at 800 nm, 76-MHz repetition rate
2. Two automated-tuning Ti:Sapphire laser: 700-1030-nm wavelength, 75-fs pulse duration, 3-W average power at 800 nm, 76-MHz repetition rate
3. Yb:fiber laser: 1030-nm wavelength, 300-fs pulse duration, 4-W average power, 6-MHz repetition rate
4. Yb-fiber laser driven optical parametric amplifier: 1330-nm wavelength, 75-fs pulse duration, 250-mW average power, 1 MHz repetition rate

5. Er:fiber laser: 1550-nm wavelength, 500-fs pulse duration, 2-W average power, 0.25 – 5 MHz repetition rate; this light is Raman shifted in a photonic crystal rod to 1700-nm wavelength, with ~50-mW average power

Commercial femtosecond laser amplifier:
Ti:Sapphire amplifier: 800-nm wavelength, 50-fs pulse duration, 1-mJ pulse energy, 1-kHz repetition rate, with separate femtosecond seed oscillator

Nonlinear microscopes:
Four home-built two-photon excited fluorescence microscopes designed specifically for in vivo animal imaging and targeted optical manipulation. Optics are designed for low-loss delivery of femtosecond pulses from any of the imaging lasers described above, compensation of dispersion to achieve shortest pulse duration at the focus, and high efficiency collection and detection of emitted fluorescence or harmonic radiation on four separate detection channels. Additional laser beams are integrated into the microscopes for sample manipulation through photochemistry and optical ablation with the amplified femtosecond laser. High-resolution three-dimensional positioning of sample is achieved through computer-controlled translation stages. The microscope uses custom data acquisition software that allows fast frame and volume imaging and rotatable line-scans. One microscope is a custom-designed hyperspectral multiphoton microscope that provides 48 channels of excitation/emission information at each voxel in a three-dimensional image. One four-channel system is additionally capable of fast scanning with resonant scanners enabling approximately 8x faster image acquisition. The 1,300 and 1,700 nm imaging sources are used to drive three-photon excited fluorescence, which enables imaging deeper into scattering samples than conventional two-photon excitation.

Conventional, fluorescence microscope:
Zeiss Examiner.D1 with bright-field and epifluorescence imaging, cooled CCD camera.

Histology:
Cryotome for sectioning tissue and facilities for immunohistology and staining.
Cell culture:

BSL II cell culture hood, mammalian and bacterial incubators, desktop centrifuges, ultracentrifuge, qPCR machine, inverted microscope.

Animal behavior:
Automated animal tracking camera system and software as well as appropriate mouse “mazes” to enable a broad range of behavioral testing, including locomotor function, spatial and working memory, sensory sensitivity.

Electrophysiology:
Dual setups for recording three-channel field potential with concurrent video monitoring of awake animals.

Subcutaneous Transmitter Equipment Set-Up provided by Open Source Instruments:
Octal Data Receiver
Eight Loop Antenna
LWDAQ Driver
Faraday Enclosure
Subcutaneous Transmitters for testing the system

SBIR · STTR
America's Seed Fund™
POWERED BY SBA

SBIR.gov SBC Registration

SBC Control ID:	SBC_000814955		
Company Name:	OPEN SOURCE INSTRUMENTS INC.		
Address:	130 MOUNT AUBURN ST		
City:	WATERTOWN		
State:	MA	**Zip:**	02472-3932
EIN (TIN):	141911312	**DUNS:**	046838184
Company URL:	opensourceinstruments.com		
Number of Employees:			6
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?			No
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?			0.00%

Cornell University
Office of Sponsored Programs

Cornell University
373 Pine Tree Road
Ithaca, NY 14850

Telephone: 607 255-5014
Fax: 607 255-5058
Web: www.osp.cornell.edu

Via Email

September 4, 2018

Open Source Instruments
5 Pratt Ave
Waltham, MA 02453
Attn: Kirsten Hasemi
kirsten@opensourcesinsturments.com

RE: Cornell University Letter of Commitment – Proposal titled: An Optogenetic Brain Implant with EEG Monitoring and Response for Mice

Dear Ms. Hasemi:

Attached please find a proposal submitted by Cornell University on behalf of Dr. Chris Schaffer, Associate Professor in Biomedical Engineering. Cornell is requesting funding in the amount of $59,884 for the period April 1, 2019 through March 31, 2020.

We understand that this proposal will be included as part of an application to the NIH SBIR Program under Program Solicitation PA-18-871.

If an award is made, Cornell will be pleased to enter into an agreement with Open Source Instruments with terms and conditions that are appropriate to an educational institution and consistent with Cornell's policies, including the determination by the Government's contracting officer that Cornell University's work under this Contract is considered to be Fundamental Research per DFARS 252.204-7000.

Any resulting award should be made in the legal name of Cornell University at the following address:

Office of Sponsored Programs
373 Pine Tree Road
Cornell University
Ithaca, New York 14850-2820

If you have any questions or if you need additional information, please contact Anne M Ochiai at 607-255-1050 or by email at amo68@cornell.edu

Sincerely,

Anne M Ochiai

Anne M Ochiai
Sr. Grant & Contract Officer
Ref: OSP# 88004

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Mr. First Name*: Michael Middle Name London Last Name*: Collins Suffix:

Position/Title*: Engineer
Organization Name*: OPEN SOURCE INSTRUMENTS
Department:
Division:
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: WATERTOWN
County: MASSACHUSETTS
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 02472-3932

Phone Number*: 978-335-3137 Fax Number:

E-Mail*: collins@opensourceinstruments.com

Credential, e.g., agency login: michaelcollins

Project Role*: PD/PI Other Project Role Category:

Degree Type: Master of Science Degree Year: 2014

Attach Biographical Sketch*: File Name: biosketch-Collins_final.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12704511 Funding Opportunity Number: PA-18-871 . Received Date: 2018-09-05T11:19:48.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Kevan Middle Name Sayed Last Name*: Hashemi Suffix:

Position/Title*: President
Organization Name*: OPEN SOURCE INSTRUMENTS, INC.
Department:
Division:
Street1*: 130 Mt. Auburn St
Street2:
City*: Watertown
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 02472-3932

Phone Number*: 6173353472 Fax Number:

E-Mail*: hashemi@opensourceinstruments.com

Credential, e.g., agency login: kevanhashemi

Project Role*: Other Professional Other Project Role Category: Electrical Engineer

Degree Type: MS,BS Degree Year: 1992,1987

Attach Biographical Sketch*: File Name: biosketch-Hashemi_final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: James Middle Name Last Name*: Bensinger Suffix: Ph.D

Position/Title*: Professor
Organization Name*: Brandeis University
Department: Physics
Division:
Street1*: 415 South Street
Street2: Abelson 312
City*: Waltham
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 02453-2700

Phone Number*: 617-610-4316 Fax Number:

E-Mail*: bensinger@opensourceinstruments.com

Credential, e.g., agency login: JAMESBENSINGER

Project Role*: Other Professional Other Project Role Category: Physicist

Degree Type: PhD Degree Year: 1970

Attach Biographical Sketch*: File Name: biosketch-Bensinger_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Chris Middle Name Last Name*: Schaffer Suffix: Ph.D

Position/Title*: Associate Professor
Organization Name*: Cornell University
Department: Biomedical Engineering
Division:
Street1*: B57 Weill Hall
Street2: 237 Tower Road
City*: Ithaca
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 148537202

Phone Number*: 607-342-7737 Fax Number: 607-255-7330

E-Mail*: cs385@cornell.edu

Credential, e.g., agency login: CBSCHAFFER

Project Role*: Other Professional Other Project Role Category: Neuroscientist

Degree Type: PhD Degree Year: 2001

Attach Biographical Sketch*: File Name: Schaffer_NIH_Biosketch.pdf

Attach Current & Pending Support: File Name: Schaffer_Support.pdf

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Michael Collins

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

POSITION TITLE: Scientist

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
Brandeis University	B.A.	06/2012	Economics
Northeastern University, Boston, MA	M.S.	08/2014	Electrical and Computer Engineering

A. Personal Statement

I began working for Open Source Instruments (OSI) in 2012 while an undergraduate student. I continued working for OSI on a part-time / contract basis while completing my Master's degree in electrical engineering. My work at OSI during this period focused on developing the hardware necessary to deliver optogenetic stimulation.

I worked at another research lab from 2014 to 2017, where I fulfilled a contractual obligation to the United States government in exchange for my graduate degree funding. In this period, I was promoted to “Junior Scientist” as a direct result of my research reports on the effect of underwater electronics on maritime security.

I returned to OSI in January, 2017. From January 2017 until present, I have worked 40% of my time at OSI and 60% of my time on my own company, which sells an electronic product to the automotive industry. Work on my own company takes place at the same location as OSI. With the receipt of this SBIR Phase I Grant, I will increase my OSI hours to 85% of my time and will use those hours exclusively for fulfilling this project's requirements.

This project is first and foremost a practical engineering challenge. Neuroscientists have made tremendous progress developing the basic science of optogenetics, and their progress has outpaced the development of the instruments that support their research. For instrumentation to catch up with proposed experiments, expertise in electronics is necessary.

I have designed and tested electronic products both at OSI and at my own company, Locator TT. The product I built for Locator TT is undergoing trials at a Nasdaq-traded company. The specifications of the proposed instrument have been carefully considered, and we are confident in our ability to meet

them. Mr. Hashemi has mentored my engineering career for nearly a decade. He will provide input to the major engineering decisions in this project.

For its neuroscience, expertise, OSI has worked closely with our neuroscientist customers on the development of each of our products since the company's first telemetry implant. We have already received feedback on our first optogenetic instruments from researchers at the Institute of Neurology at University College London. Moving forward with this proposal, I will be working closely with Dr. Schaffer at Cornell and his PhD student Seth Lieberman. Their input will guide all decisions that require a deeper understanding of the neuroscience or anatomy.

As PI, I will be using my experience leading product development, and I will leverage OSI's in-house expertise, our collaborator's expertise, and the network of subject matter experts that OSI has built over the past decade via our open-collaboration business model.

B. Positions and Honors

2009 - 2012	Research Assistant; High Energy Physics Electronics, Brandeis; Waltham, MA
2013 (Summer)	Research Intern; Los Alamos National Laboratory; Los Alamos, NM
2012 - 2014	Scientist; Open Source Instruments; Waltham, MA
2012 - 2014	Research Assistant; Northeastern University; Boston, MA
2014 - 2016	Junior Scientist; Centre for Maritime Research and Experimentation; La Spezia, Italy
2017 – Present	Scientist; Open Source Instruments; Waltham, MA
2017 – Present	Founder; Locator TT; Waltham, Ma

C. Contributions to Science

While employed at the Centre for Maritime Research and Experimentation (CMRE), the director of the Anti-Submarine Warfare program made me responsible for a new project related to “Civilian Monitoring Networks”. (They are NOT networks that monitor civilians; that lexicology is a poor and ambiguous choice.) My role was to apply an interdisciplinary approach to the problem and fuse the knowledge of engineers with that of ocean acousticians. I subsequently led the organization of an international workshop on the subject and authored several reports. I used computational modeling, signal processing, and assessment of electronics to study this subject and its implication for warfare. I am not authorized to discuss the work openly beyond referencing the papers listed on CMRE's library webpage. Eventually, this work may contribute to marine biology data collection and processing methods.

1. Collins, Michael and Kevin D. LePage. “Civilian Monitoring Network Risks and Recommendations.” Full Report. La Spezia: STO-CMRE, 2017.
2. Collins, Michael. “Civilian Monitoring Network Workshop Outcomes.” Full Report. La Spezia: STO-CMRE, 2017.
3. Collins, Michael. “Taxonomy of civilian monitoring networks.” Memorandum Report. CMRE-MR-2015-019. La Spezia: STO-CMRE, 2016.
4. Collins, Michael. “Civilian Monitoring Network (CMN) high level report.” Memorandum Report. CMRE-MR-2015-018. La Spezia: STO-CMRE, 2016.

While a Master's student, I studied Nuclear Quadrupole Resonance (NQR) and demonstrated that biological tissue does not have a significant impact on its performance as an explosives detection technology.

5. Collins, Michael L. “Detecting body cavity bombs with nuclear quadrupole resonance.” MS Thesis. Boston: Northeastern University, 2014.

While at Open Source Instruments, I worked on the efficient coupling of light into optical fibers. One of the largest problems that optogenetic instruments face is their ability to deliver an adequate amount of optical power into neural tissue to activate the photosensitive protein channels. Equipment tethered to benchtop power supplies solves this problem by using a very powerful, but very inefficient mechanism for coupling light from an LED or laser source into neural tissue. In the case of a wireless instrument, we must be able to deliver the same amount of optical power with a much smaller electrical energy budget. To meet this requirement, I came up with several innovations in conjunction with Mr. Hashemi.

The first innovation was to have custom optical fibers built out of special glass with a much higher index of refraction than are typically commercially available.

The second innovation was to stop using commercially available LEDs in conventional packages and instead purchase the dies directly from the manufacturer and then mount them on our own substrate with wire bonding and eutectic bonding equipment. This gives a more favorable angle of illumination for light entering the optical fiber than was possible with off-the-shelf LEDs.

The third innovation was to design optical fibers that are tapered at the end where light emerges into the brain. The taper minimizes the amount of opaque scar tissue that the brain develops, thus requiring a lower amount of optical power. The taper also increases the surface area from which light escapes the fiber, increasing the total volume of neural tissue that receives optical power above the threshold necessary for opsin activation. Without the taper, the same total amount of optical power would be delivered, but most of that power would go into a smaller volume, and so less total volume of brain tissue would meet the threshold of activation.

These innovations may become considered contributions to science upon testing of the proposed MS-ISL *in vivo.* They will be fundamental to the new device's ability to operate in chronic experiments off of a battery so small that it's surgically implanted in a mouse.

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

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Kevan Hashemi

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

POSITION TITLE: Electrical Engineer, Adjunct Faculty, Brandeis University

President and Founder, Open Source Instruments

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
Cambridge University, Cambridge, UK	B.S.	05/01/87	General Engineering
Cornell University, Ithaca, NY	M.S.	05/01/92	Electrical Engineering

A. Personal Statement

I started Open Source Instruments to make electronic devices for research scientists that I had come to know through my work and education. This application for the BRAIN Initiative SBIR is a continuation of more than a decade of personal commitment to my project.

In 2004, James Bensinger and I were asked to build several hundred thousand dollars worth of opto-electronic devices for a particle detectors at the CERN laboratory in Geneva. Thus, we founded Open Source Instruments Inc. In 2005, a friend from Cambridge, Matthew Walker, who is head of the Institute of Neurology (ION) at University College London, asked me to design an effective, wireless device to monitor EEG in rats. Subsequently, in 2012, I was asked by Dr. Walker's colleague, Dimitri Kullmann at ION, to design a wireless device to provide both light stimulation and EEG monitoring in rats. This application for the BRAIN Initiative SBIR is a continuation of 13 years of successful development. I am confident this is the next, best step in our journey since we have received dozens of requests from our current customers for a mouse-sized optogenetic device.

I work closely with our customers to help them bring their research to completion. I wrote the event-detection software they use to analyze tens of thousands of hours of EEG recordings they collect. I am co-author on half a dozen research papers because of my contribution to their analysis and the planning of their experiments. There are few electrical engineers who can design and build radio-frequency communication systems themselves without using commercial, off-the-shelf components. As a result, my designs are adapted to the specifics of the project need. For example, they have longer battery life.

As founder and Chief Executive of Open Source Instruments, I bring to this proposed project a well-established and proven group of technical staff, including two radio-frequency experts, myself and Michael Collins, and three experts in optics: myself, Michael Collins, and James Bensinger. I have been working for Professor Bensinger at Brandeis University, where I am an adjunct faculty member, for twenty-five years, designing and manufacturing opto-electronics for High Energy Physics experiments. Michael Collins started working for Prof. Bensinger and I at Brandeis University in 2010. He now works for us at Open Source Instruments.

All of Open Source Instruments' telemetry devices are designed to specifications that evolve from open discussion with the customers. This open discussion is possible only because we do not keep our methods secret. Our designs and software are free for all to look at, just as the experimental methods of our customers are free for all to read. Our open relationship with our customers is unique among manufacturers of telemetry device. This relationship allows us to design instruments that are of real value to neuroscientists. The telemetry system we provided to ION in 2010 has allowed ION's experimental epilepsy group to accelerate and expand its research. Over the past eight years, a dozen other laboratories around the world have started using the same system. Their growing publication record speaks for the reliability and fidelity of the EEG recordings they obtain with our equipment.

The wireless optogenetics market has seen several ingenious but impractical devices come and go. Our proposed device is different. Its specification is the result of several years of discussion with the scientists who want to use it. With our experience designing and supporting implantable devices for neuroscience, our mouse-sized optogenetics device will be both practical and valuable. Working on this project is the next best step for me and Open Source Instruments in our journey of building effective research tools for scientists.

B. Positions and Honors

2004-present	President, Open Source Instruments, Watertown and Waltham, MA
1995-present	Electrical Engineer and Adjunct Faculty, Martin A. Fisher School of Physics, Brandeis University, Waltham, MA
1992-1994	Electrical Engineer, Superconducting Super Collider, Harvard University, Cambridge, MA
1987-1989	Electrical Engineer, Meta Machines, Ltd, Abingdon, Oxfordshire, UK

C. Contributions to Science

1. Nowadays, laser rangefinders are standard equipment for precision survey work. The rangefinder measures the distance to a target directly. A rangefinder eliminates the need for rulers and reference bars, and greatly accelerates the survey process. In the period 1990-2010, a dozen research groups around the world were working on their own laser rangefinder designs. I was part of one such group at the Harvard High Energy Physics Laboratory. Our purpose was to figure out how to build a rangefinder accurate to a tenth of a millimeter that could be manufactured for a few thousand dollars and installed by the hundreds in the Superconducting Super-Collider (SSC) in Texas. We built a successful prototype, which we describe in our paper "*Sources of Error in a Laser Rangefinder*" (Hashemi et al. 1994 Review of Scientific Instruments. 65. 3165 - 3171. 10.1063/1.1144545). Our work formed part of the successful global effort that produced the economical and effective laser rangefinders available today. We like to think that we could have gone on to produce thousands of such rangefinders for the SSC, but funding for the SSC was cancelled in 1994, and our work on that device had to stop.

2. The ATLAS experiment is a particle detector 40 m long and 20 m in diameter that sits 100 m below the ground in Geneva, Switzerland, at the CERN laboratory. I worked with High Energy Physics group at Brandeis University from 1995 to 2010 on the design, manufacture, and installation of the opto-

electronic system that monitors deformations of the end-caps of this detector. Accurate measurement of the absolute position of the muon-detectors in the ATLAS end-caps is essential to the accurate measurement of the momentum and trajectory of muons passing through its magnetic field. There were several opto-electronic devices competing for installation in this monitoring system at the time we joined the collaboration in 1995. Our design was the Brandeis CCD Angle Monitor (BCAM), a radiation-resistant, solid-state camera with a robust and stable chassis that we could calibrate so as to provide absolute measurement accuracy of better than fifty microradians, and relative accuracy of five microradians (. At a range of ten meters, these angular accuracies translate to half a millimeter and a twentieth of a millimeter. The BCAM won through as the best device for the job, and we installed thousands of them in the ATLAS detector. Subsequently, the remaining three particle detectors operating on the Large Hadronic Collider at CERN asked to be supplied with BCAMs for their own alignment systems, and we founded Open Source Instruments Inc. to manufacture these devices. We report on the performance of the BCAM in the ATLAS alignment system in "*The Optical Alignment System of the ATLAS Muon Spectrometer Endcaps*" (Amelung et al. 2008 ATLAS Muon Note ATL-MUON-PUB-2008-003), a technical paper published by the ATLAS collaboration. I personally designed all the electronics used in the BCAM, as well as wrote the core data acquisition software, image analysis software, and software that takes calibration measurements and calculates the parameters that define each BCAM camera. My colleague, James Bensinger, designed the precision roll-cage that permits us to obtain the necessary calibration measurements. As members of the ATLAS Collaboration, we are co-authors on the landmark physics paper "*Observation of a new particle in the search for the Standard Model Higgs boson with the ATLAS detector at the LHC*" (Aad et al. 2012, Physics Letters B, Volume 716, Issue 1, Pages 1-29), along with several thousand other people. In the past ten years we have designed and built six new versions of the BCAM, and these have been installed in half a dozen new detectors in Europe, the United States, South America, and even India.

3. I began my collaboration with Dr. Matthew Walker of the Institute of Neurology (ION) at University College London (UCL) in 2005. Our purpose was to design and build a battery-powered device that could be implanted subcutaneously in a rat and record high-fidelity EEG for two months. We produced such a device in 2010 ("*A Novel Telemetry System for Recording EEG in Small Animals*" Chang et al. 2011, Journal of Neuroscience Methods, 201(1): 106-115). These rat-sized implants made possible an entire series of experiments at ION in the past eight years. Now that researchers could acquire tens of thousands of hours of high-fidelity EEG from freely-moving animals, they had to figure out how to search through the recordings automatically to count seizures, spikes, and other unusual events. I developed our Event Classifier software for this purpose, and ION describes their use of the Event Classifier in the Methods section of "*Optogenetic and Potassium Channel Gene Therapy in a Rodent Model of Focal Neocortical Epilepsy*" (Wykes et al. 2012, Science Translational Medicine, DOI: 10.1126/scitranslmed.3004190), a paper in which I am a co-author for my contribution to their analysis. I am likewise a co-author on two other published neuroscience papers for similar contributions to EEG analysis (Brown et al. 2018, eNeuro, ENEURO.0426-17.2018; Wright et al. 2015, BRAIN Journal of Neurology, Oxford University Press, 138(9)) and another submitted for publication (Snowball et al. 2018 bioRxiv 298588; doi: 10.1101/298588;). These papers do not include the work done by other users of our subcutaneous transmitters by customers who read our documentation and put our analysis to use without any significant contribution on my part.

4. I began my collaboration with Dr. Louise Upton of the Department of Physiology, Oxford University, in 2012. Our purpose was to develop a subcutaneous transmitter small enough to be implanted in mice. In 2015 Dr. Upton and her group completed their first study with the mouse-sized implants, as reported in "*Epileptogenic effects of NMDAR antibodies in a passive transfer mouse model*" (Wright et al. 2015, BRAIN Journal of Neurology, Oxford University Press, 138(9)).

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

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

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

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

POSITION TITLE: Professor of Physics

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
Bucknell University	BSc	05/01/63	Physics
University of Wisconsin	PhD	06/01/70	Physics

A. Personal Statement

I am pleased to be part of this application for an PA-18-871 award. The technical people in this group are the same people that designed our profitable line of implantable telemetry devices for Open Source Instruments. Prior to that, the team worked together at Brandeis University to design, manufacture, and install the opto-electronic system that monitors the deformations of the ATLAS particle detector at CERN.

I am a minority owner of Open Source Instruments Inc., an investor in the company, and its acting treasurer. The company started with a project which grew out of research at Brandeis University, where I work with Kevan Hashemi, Open Source Instruments Founder and President. I met Kevan when we were both working on an experiment at CERN in 1993 while Kevan was at Harvard. When the funding on the Harvard project ended, I took the opportunity to hire Kevan at Brandeis, where he has been since. We have worked together for 23 years.

I bring to this project my knowledge of physics and in particular that of the propagation of light through optical fibers. In any development like this, there are always surprising physical phenomena that arise as obstacles to the development. My contribution to the development efforts at Open Source Instruments is often that of studying and understanding such surprises, a function for which my background in physics is of great value.

I will be working part-time at Brandeis University starting next year, allowing me to spend more time working on research at Open Source Instruments. I look forward to working on independent projects with a talented group of scientists. I very much hope to be able to work on efficient light injection and fiber-optic delivery for a mouse-sized ISL.

"Reference Bars for the Alignment of the ATLAS Muon Spectrometer" C. Amelung, J.R. Bensinger, et al., Nuclear Instruments and Methods A555, 36-47 (2005).

"The Optical Alignment System of the ATLAS Muon Spectrometer Endcaps," C. Amelung, et al., JINST 3:P11005, 2008.

"The ATLAS Experiment at the CERN Large Hadron Collider," The ATLAS Collaboration, JINST 3:S08003, 2008.

"System test of the ATLAS muon spectrometer in the H8 beam at the CERN SPS," The ATLAS Muon Collaboration, Nucl. Instrum. Meth. A593:232-254,2008. May 2008.

"Study of the ATLAS MDT spectrometer using high energy CERN combined test beam data," C. Adorisio, et al., Nucl. Instrum. Meth. A598:400-415, 2009.

B. Positions and Honors

1970 – 73: Instructor/Assistant Professor, University of Pennsylvania
1973 – 74: Research Associate, Brandeis University
1974 – 80: Assistant Professor, Brandeis University
1980 – 89: Associate Professor, Brandeis University
1989 – Present: Professor, Brandeis University
1996 – 2000: Chair, Brandeis University Physics Department

C. Contributions to Science

1. "Measurement of transverse energy--energy correlations in multi-jet events in pp collisions at $\sqrt{s}$ = 7 TeV using the ATLAS detector and determination of the strong coupling constant $\alpha_s(m_Z)$" ATLAS Collaboration, Physics Letters B 750 (2015) 427-447.
2. "Measurement of the branching ratio $\Gamma(\Lambda^0_b \to \psi(2S)\Lambda^0)/\Gamma(\Lambda^0_b \to J/\psi\Lambda^0)$ with the ATLAS detector" ATLAS Collaboration, Physics Letters B 751 (2015) 63-80.
3. "Summary of the searches for squarks and gluinos using $\sqrt{s}$ = 8 TeV pp collisions with the ATLAS experiment at the LHC" ATLAS Collaboration, JHEP 10 (2015) 054.
4. "Search for photonic signatures of gauge-mediated supersymmetry in 8 TeV pp collisions with the ATLAS detector" ATLAS Collaboration, Phys. Rev. D 92 (2015) 072001.
5. "Determination of the top-quark pole mass using tt-bar + 1-jet events collected with the ATLAS experiment in 7 TeV pp collisions" ATLAS Collaboration, JHEP 10 (2015) 121,

Collaborators & Other Affiliations (2000-present)

ATLAS Collaboration

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

Graduate and Postdoctoral Advisors

Alvin Erwin, University of Wisconsin.
Walter Selove, University of Pennsylvania

Thesis and Postdoc Sponsor

Postdoctoral (3 total):

Saminder Dhaliwal 2014-Present

Dmitri Kotchetkov	2005-2007
David Dagenhart	2001-2009

PhD students (9 total):

Keith Zengel	2015
Laurel Coffey	2014
Serdar Gozpinar	2012
Dan Pomeroy	2012
Scott Aefsky	2011
David Clark	2010
Natasa Kravchenko	2008
Hongquan Niu	2003

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BIOGRAPHICAL SKETCH

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NAME: Schaffer, Chris B.

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

POSITION TITLE: Associate Professor; Associate Dean of the Faculty

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 Florida, Gainesville, FL	BS	12/1995	Physics
Harvard University, Cambridge, MA	PHD	06/2001	Physics
Univ. of California, San Diego, La Jolla, CA	Postdoctoral Fellow	06/2002	Biophysics and Neuroscience

A. Personal Statement

My lab develops and uses advanced optical techniques to observe and manipulate in vivo biological systems, with the goal of constructing a microscopic-scale understanding of normal and disease-state physiological processes in the central nervous system. The scientific questions we address center principally on elucidating the cellular-scale interactions that lead to brain cell dysfunction in neurological diseases. We develop novel optical methods that enable us to attack these problems in ways not previously possible, and because many of our research questions involve interactions among different components of an organism (e.g. effect of altered blood flow on brain cell health) we focus almost exclusively on in vivo approaches. We study animal models of a variety of neurological diseases, including microvascular stroke, Alzheimer's disease, spinal cord injury, and epilepsy. In exciting new work, we are beginning to elucidate the pathways by which cortical microvascular dysfunction interacts with and exacerbates Alzheimer's disease. We have also recently developed capabilities for long-term in vivo imaging in the mouse spinal cord, opening the door to optical imaging studies of normal and diseased cell dynamics in the spinal cord. I am also active in developing novel educational strategies to teach science as a dynamic process for discovery. These approaches are used in outreach settings in middle and high-school science classes as well as in my undergraduate and graduate level courses. I also have a strong interest in science policy and recently spent a one-year sabbatical in Washington, DC, working as a science policy fellow for Senator Edward Markey in the United States Congress. I continue to be active in science policy, including through a course I teach on this topic. In recent work, we demonstrated a laser-based surgical therapy that blocks the propagation of focally initiated seizures in rodents [1]. In ongoing work to evaluate the long-term efficacy of that approach, we have established a robust chronic rodent model of focal epilepsy in our lab and it is this model that we would use for the proposed testing of the implanted wireless EEG/optogenetic feedback device from Open Source Instruments

[1] Nagappan S, Liu L, Fetcho R, Nguyen J, Nishimura N, Radwanski R. In vivo femtosecond laser sub-surface cortical microtransections attenuate acute rat focal seizures. Cerebral Cortex 2018 (in press).

B. Positions and Honors

Positions and Employment

2002 - 2005 Assistant Project Scientist and La Jolla Interfaces in Science Fellow, Dept. of Physics, Univ. of California at San Diego, Advisors: David Kleinfeld and Patrick Lyden

2006 - 2011 Assistant Professor, Department of Biomedical Engineering, Cornell University

2011 - Associate Professor, Meinig School of Biomedical Engineering, Cornell University

2012 - 2013 OSA/SPIE Arthur H. Guenther Congressional Science Policy Fellow, Office of Senator Edward J. Markey, United States Congress

2013 - 2016 Director of Graduate Studies, Graduate Field of Biomedical Engineering, Cornell University

2014 - Associate Professor, Dept. of Neuroscience, Brain and Mind Research Institute, Weill Cornell Medical College

2016 - Associate Dean of the Faculty, Cornell University

Other Experience and Professional Memberships

2002 - 2006	Vice-Chair (02-04) and Chair (04-06), Ultrafast Optical Phenomena Technical Group, Optical Society of America Science and Engineering Council and Annual Meeting committee
2002 - 2007	Chair, Commercial and Biomedical Applications of Ultrafast Lasers SPIE's Photonics West
2005 - 2007	Chair, Optical Microscopy and OCT Technical Group, Optical Society of America Science and Engineering Council and Annual Meeting committee
2006 - 2010	Vice-Chair (06-07) and Chair (07-10), Biomedical Optics, OSA Board of Meetings
2009 - present	Reviewer, National Science Foundation, MRI-R2 (2009, 2010) and BISH (2009) panels; ad-hoc reviewer
2009 - 2014	Reviewer, Alzheimer's Association
2010 - 2010	Reviewer, Congressionally Directed Medical Program: Epilepsy panel (2010)
2011 - 2012	Working Group Member, NIH/NINDS 2012 Stroke Progress Review Group
2012 - 2013	Program Committee Member, Brain 2013, XXVIth International Symposium on Cerebral Blood Flow, Metabolism, and Function
2014 - present	Associate Editor, Neurophotonics
2016 - present	Associate Editor, Optica

Honors

1995	Outstanding Four Year Scholar, University of Florida, awarded to two graduates out of a class of 4000 for academic excellence in undergraduate studies
1996	National Defense Science and Engineering Graduate Fellowship, US Department of Defense
1996	Apker Award, American Physical Society, awarded to one undergraduate from a Ph.D.-granting institution nationally for achievements in research and academics
2001	New Focus Student Award, Optical Society of America, recognizes Ph.D. students for research excellence, presentation skills, and leadership in the optics community
2002	La Jolla Interfaces in Science Post-Doctoral Fellowship, Burroughs Wellcome fund
2008	Dorothy G. Swanson Excellence in Teaching Award, College of Engineering at Cornell University, highest award for teaching excellence in the College
2009	Biomedical Engineering Teaching Award, American Society for Engineering Education, national award for contributions to biomedical engineering education by a junior faculty member
2010	Zellman Warhaft Commitment to Diversity Faculty Award, College of Engineering at Cornell, for outstanding involvement in and support of diversity activities in the College
2012	Arthur H. Guenther Congressional Science Policy Fellowship, OSA and SPIE, supports one year of service as a science policy advisor in the United States Congress through a program administered by AAAS
2014	James M. and Marsha D. McCormick Award for Outstanding Advising of First-Year Engineering Students, College of Engineering at Cornell, highest award for advising in the college
2016	Mr. and Mrs. Richard F. Tucker Excellence in Teaching Award from the College of Engineering at Cornell University, highest award for teaching excellence in the College

C. Contribution to Science

1. When tightly-focused into biological samples or tissue, femtosecond duration laser pulses can produce micrometer-scale disruption, while causing minimal collateral damage to structures around the targeted region. Essentially, the laser acts as a light scalpel that can cut with sub-cellular precision deep inside a sample without affecting the overlying tissue. As a graduate student, I studied the laser-material interactions that enable this sub-surface disruption. As a post-doc, I worked on the use of this capability to trigger the clotting or hemorrhage of small blood vessels in rodent cortex, producing an excellent animal model of microvascular stroke (which my and other labs continue to use today). In my lab at Cornell, we have further studied the use of this laser scalpel to create a transient pore in a cell membrane to introduce foreign DNA into a targeted cell. We have also pioneered the use of this laser scalpel to produce sub-surface cuts in the cortex, which we are now using to explore laser-based surgical strategies to inhibit the propagation of focal epileptic seizures.

a. Schaffer CB, Brodeur A, García JF, Mazur E. Micromachining bulk glass by use of femtosecond laser pulses with nanojoule energy. Opt Lett. 2001 Jan 15;26(2):93-5. PubMed PMID: 18033517.

b. Nishimura N, Schaffer CB, Friedman B, Tsai PS, Lyden PD, et al. Targeted insult to subsurface cortical blood vessels using ultrashort laser pulses: three models of stroke. Nat Methods. 2006 Feb;3(2):99-108. PubMed PMID: 16432519.

c. Nguyen J, Ferdman J, Zhao M, Huland D, Saqqa S, et al. Sub-surface, micrometer-scale incisions produced in rodent cortex using tightly-focused femtosecond laser pulses. Lasers Surg Med. 2011 Jul;43(5):382-91. PubMed PMID: 21674543.

d. Davis AA, Farrar MJ, Nishimura N, Jin MM, Schaffer CB. Optoporation and genetic manipulation of cells using femtosecond laser pulses. Biophys J. 2013 Aug 20;105(4):862-71. PubMed PMID: 23972838; PubMed Central PMCID: PMC3752125.

2. Even subtle alterations in cerebral blood flow can impact the health and function of brain cells and are linked to cognitive decline and dementia. My lab has made significant strides toward understanding how blood flow is altered in the brain by microvascular occlusions. As a post-doc, I helped develop optical tools to occlude targeted brain microvessels, providing a much-needed animal model for small strokes. I contributed to studies that used this model to quantify the blood flow changes that result from the occlusion of brain arterioles and capillaries. In my lab at Cornell, we have completed this story by quantifying the flow changes that result from venule occlusions and by examining the role of active vascular regulation in blood flow rerouting after a vessel occlusion. This work was essential to understanding the immediate impact of microvascular occlusion on the brain (altered blood flow) and set the stage for studies of the effect of these lesions on the health and function brain cells, now being conducted in my and other labs using the approaches we developed.

a. Schaffer CB, Friedman B, Nishimura N, Schroeder LF, Tsai PS, et al. Two-photon imaging of cortical surface microvessels reveals a robust redistribution in blood flow after vascular occlusion. PLoS Biol. 2006 Feb;4(2):e22. PubMed PMID: 16379497; PubMed Central PMCID: PMC1324794.

b. Nishimura N, Schaffer CB, Friedman B, Lyden PD, Kleinfeld D. Penetrating arterioles are a bottleneck in the perfusion of neocortex. Proc Natl Acad Sci U S A. 2007 Jan 2;104(1):365-70. PubMed PMID: 17190804; PubMed Central PMCID: PMC1765467.

c. Nishimura N, Rosidi NL, Iadecola C, Schaffer CB. Limitations of collateral flow after occlusion of a single cortical penetrating arteriole. J Cereb Blood Flow Metab. 2010 Dec;30(12):1914-27. PubMed PMID: 20842163; PubMed Central PMCID: PMC3002886.

d. Nguyen J, Nishimura N, Fetcho RN, Iadecola C, Schaffer CB. Occlusion of cortical ascending venules causes blood flow decreases, reversals in flow direction, and vessel dilation in upstream capillaries. J Cereb Blood Flow Metab. 2011 Nov;31(11):2243-54. PubMed PMID: 21712834; PubMed Central PMCID: PMC3210348.

3. Recent clinical evidence suggests that small hemorrhages from the rupture of microvessels in the brain are linked to increased risk of neurodegenerative diseases, as well as to more precipitous cognitive decline with age. However, it remains unclear how severely and by what mechanism a microhemorrhage causes death or dysfunction in brain cells. This is due, in part, to a lack of good animal models of microhemorrhage. We used in vivo imaging coupled with our unique animal model of microvascular hemorrhage to study how such lesions affect the function and structure of neurons and other brain cells. We found, surprisingly, that microhemorrhages do not lead to structural degeneration or long-term functional impairment in neurons, but rather cause a rapid inflammatory response in microglia that is sustained in the vicinity of the microhemorrhage over weeks after the lesion. This data suggests that chronic, local inflammation may underlie the brain dysfunction that results from microhemorrhages rather than direct neuronal damage, a hypothesis we are currently pursuing. We have also used this hemorrhage model in collaborative studies of the impact of anticoagulants and thrombolytics on brain hemorrhage size.

a. Rosidi NL, Zhou J, Pattanaik S, Wang P, Jin W, et al. Cortical microhemorrhages cause local inflammation but do not trigger widespread dendrite degeneration. PLoS One. 2011;6(10):e26612. PubMed PMID: 22028924; PubMed Central PMCID: PMC3197572.

b. Cianchetti FA, Kim DH, Dimiduk S, Nishimura N, Schaffer CB. Stimulus-evoked calcium transients in somatosensory cortex are temporarily inhibited by a nearby microhemorrhage. PLoS One. 2013;8(5):e65663. PubMed PMID: 23724147; PubMed Central PMCID: PMC3665593.

c. Lauer A, Pfeilschifter W, Schaffer CB, Lo EH, Foerch C. Intracerebral haemorrhage associated with antithrombotic treatment: translational insights from experimental studies. Lancet Neurol. 2013 Apr;12(4):394-405. PubMed PMID: 23518332; PubMed Central PMCID: PMC3702044.

d. Nishimura N, Schaffer CB. Big effects from tiny vessels: imaging the impact of microvascular clots and hemorrhages on the brain. Stroke. 2013 Jun;44(6 Suppl 1):S90-2. PubMed PMID: 23709743; PubMed Central PMCID: PMC3862170.

4. Long-term in vivo imaging in the cortex of mice has become a powerful tool for dissecting the cellular interactions that underlie normal and disease state physiological processes. We have recently worked to develop animal surgical preparations and imaging strategies that would allow such in vivo imaging approaches to be used in the spinal cord of mice. The surgical preparation we developed was the first to enable long-term optical access to the murine spinal cord, and we used this preparation to examine the heterogeneity of axon dieback and the inflammatory response after a mild spinal cord injury. We have further developed label-free optical methods for visualizing myelin in the spinal cord, and recently used our animal preparation to examine the impact of vascular occlusions on spinal cord blood flow. Recently, we contributed to studies showing that using higher order nonlinear processes for imaging allows deeper penetration into scattering tissue, such as the white matter tracks on the dorsal spinal cord. Taken together, we are moving toward the capability to directly image structure and function (e.g. neural activity) of all cellular constituents through most of the spinal cord over time in wildtype or disease-model mice.

a. Farrar MJ, Wise FW, Fetcho JR, Schaffer CB. In vivo imaging of myelin in the vertebrate central nervous system using third harmonic generation microscopy. Biophys J. 2011 Mar 2;100(5):1362-71. PubMed PMID: 21354410; PubMed Central PMCID: PMC3043202.

b. Farrar MJ, Bernstein IM, Schlafer DH, Cleland TA, Fetcho JR, et al. Chronic in vivo imaging in the mouse spinal cord using an implanted chamber. Nat Methods. 2012 Jan 22;9(3):297-302. PubMed PMID: 22266542; PubMed Central PMCID: PMC3429123.

c. Horton NG, Wang K, Kobat D, Clark CG, Wise FW, et al. In vivo three-photon microscopy of subcortical structures within an intact mouse brain. Nat Photonics. 2013 Mar 1;7(3)PubMed PMID: 24353743; PubMed Central PMCID: PMC3864872.

d. Farrar MJ, Rubin JD, Diago DM, Schaffer CB. Characterization of blood flow in the mouse dorsal spinal venous system before and after dorsal spinal vein occlusion. J Cereb Blood Flow Metab. 2015 Jan 7;PubMed PMID: 25564237.

5. My lab has been an effective and enabling collaborator on a number of different studies. We have provided expertise in animal models and in in vivo optical imaging that has enabled critical aspects of high-profile papers on diverse topics such as the role of arterial stiffening in arthrosclerosis development, the genetic and cellular mechanisms underlying the formation of arteriovenous malformations, the nature of and mechanisms for blood flow change during epileptic seizures, and the use of functionalized liposomes to target and kill circulating tumor cells in the blood stream. This willingness to adapt and apply our expertise in areas outside the lab's primary scientific focus has become a true hallmark of the lab.

a. Zhao M, Nguyen J, Ma H, Nishimura N, Schaffer CB, et al. Preictal and ictal neurovascular and metabolic coupling surrounding a seizure focus. J Neurosci. 2011 Sep 14;31(37):13292-300. PubMed PMID: 21917812; PubMed Central PMCID: PMC3191875.

b. Huynh J, Nishimura N, Rana K, Peloquin JM, Califano JP, et al. Age-related intimal stiffening enhances endothelial permeability and leukocyte transmigration. Sci Transl Med. 2011 Dec 7;3(112):112ra122. PubMed PMID: 22158860; PubMed Central PMCID: PMC3693751.

c. Mitchell MJ, Wayne E, Rana K, Schaffer CB, King MR. TRAIL-coated leukocytes that kill cancer cells in the circulation. Proc Natl Acad Sci U S A. 2014 Jan 21;111(3):930-5. PubMed PMID: 24395803; PubMed Central PMCID: PMC3903223.

d. Murphy PA, Kim TN, Huang L, Nielsen CM, Lawton MT, et al. Constitutively active Notch4 receptor elicits brain arteriovenous malformations through enlargement of capillary-like vessels. Proc Natl Acad Sci U S A. 2014 Dec 16;111(50):18007-12. PubMed PMID: 25468970; PubMed Central PMCID: PMC4273347.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/myncbi/chris.schaffer.1/bibliography/40950403/public/?sort=date&direction=ascending

SUPPORT

Schaffer, Chris B.

School of Biomedical Engineering

Cornell University

Associate Professor; Associate Dean of the Faculty

D. Research Support

Ongoing Research Support

R01 AG049952-01 Schaffer (PI) 05/15/2015 – 04/30/2020
National Institute on Aging (NIA)
Stalled capillary flow: A novel mechanism for hypoperfusion in Alzheimer's disease
This proposal tests the idea that reactive oxygen species produced in the Alzheimer brain leads to upregulation of cell adhesion markers in the endothelium, causing leukocyte adhesion in a subset of brain capillaries, thereby causing a global decrease in blood flow.
Role: PI

PR151579P1 Wang (PI) 07/1/2016 - 06/30/2021
Congressionally Directed Medical Research Program
Rbpj And Ephrinb2 As Molecular Targets To Treat Brain Arteriovenous Malformation In Notch4-Induced Mouse Models
The major goals of this project: (1) Determine the effects of inhibiting Rbpj on the regression of the Notch4* BAVM model; (2) Determine the roles for ephrin-B2 as a therapeutic target in the Notch4* BAVM model; (3) Reveal the mechanism of AVM regression by time-lapse live imaging in the Notch4* BAVM model.
Role: Co-PI

R21 NS096669 Schaffer (PI) 10/01/2016 – 09/30/2018
National Institutes of Health
Three Photon Imaging Of Neural Activity In The Spinal Cord Of Awake Mice
Neurons in the spinal cord form circuits that control rhythmic motions such as walking and running. Despite the importance of understanding how these circuits work and how they fail after spinal cord injury or in diseases such as amyotrophic lateral sclerosis, there is no existing approach to directly measure the patterns of activity across a large ensemble of spinal cord neurons in awake, moving animals.
Role: PI

DOH01-C32094GG Schaffer (PI) 01/01/2017 – 12/31/2018
New York State Department of Health
Imaging neural activity in the spinal cord of awake mice after spinal cord injury
This project will establish the capability to image patterns of neural activity in the spinal cord of awake, behaving mice with and without spinal cord injury and demonstrate the utility of this approach in a study of the motion-correlated activity patterns of one class of spinal cord neurons.
Role: PI

A2017488S Schaffer (PI) 07/01/2017 – 06/30/2019
Brightfocus Foundation
Blocking neutrophil adhesion to improve brain blood flow in Alzheimer's disease
We propose to screen drugs that interfere with leukoctye adhesion and that are proven to be safe in humans to find compounds that block the capillary stalling phenomena we found to be responsible for reduced brain blood flow and some cognitive dysfunction in AD mouse models.
Role: PI

1707312 Xu (PI) 09/01/2017 – 08/31/2022
National Science Foundation

NeuroNex Technology Hub: Optical technologies for large scale, noninvasive recording of neural activity
This center grant focuses on developing advanced optical imaging techniques, including three-photon excited fluorescence microscopy, to image patterns of neural activity with single-cell resolution and across large areas of the central nervous system in multiple animal models during complex behavioral tasks.
Role: Co-PI

R01 EB002019 Wise (PI) 09/01/2016 – 08/31/2020
National Institutes of Health
Laser and microscope development for multicolor nonlinear imaging deep in tissue
The major goals are the development of practical sources of high-energy ultrashort light pulses, along with novel microscope designs that will dramatically increase penetration depth and information density, which will enable novel studies of health and disease in animal models.
Role: Co-I

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: 0468381840000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: OPEN SOURCE INSTRUMENTS, INC.

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Michael		Collins		PD/PI		12.0			70,000.00	28,000.00	98,000.00
2 .		Kevan		Hashemi		Electrical Engineer, Design & Production Integration		4.0			16,000.00	1,224.00	17,224.00
3 .		James		Bensinger		Physicist		1.0			4,000.00	306.00	4,306.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **119,530.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	3.0			6,000.00	459.00	6,459.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**6,459.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**125,989.00**

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

Funding Opportunity Number: PA-18-871 . Received Date: 2018-09-05T11:19:48.000-04:00

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

ORGANIZATIONAL DUNS*: 0468381840000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: OPEN SOURCE INSTRUMENTS, INC.

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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)	980.00
2. Foreign Travel Costs	
Total Travel Cost	**980.00**

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*: 0468381840000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: OPEN SOURCE INSTRUMENTS, INC.

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,500.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	59,884.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**99,384.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**226,353.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . rent			5,400.00
2 . General Supplies			180.00
3 . Accountant			4,000.00
4 . Workers Compensation Insurance			500.00
		Total Indirect Costs	**10,080.00**

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)	**236,433.00**

J. Fee	Funds Requested ($)*
	8,000.00

K. Total Costs and Fee	Funds Requested ($)*
	244,433.00

L. Budget Justification*	File Name: Budget_Justification_Final.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification

A. Senior/Key Personnel
Salaries and wages are based on standard rates/salaries which are comparable to others doing similar effort.

Fringe benefit rate of 40% is requested for employees whose primary role will be with Open Source Instruments. For other contributing personnel we request a fringe benefit rate of 7.65% for FICA.

B. Other Personnel
Salaries and wages are based on standard rates/salaries which are comparable to others doing similar effort.

Funds are requested to provide salary support for the technician and treasurer for 6 months time, or half their duties during the period of the grant.

A fringe benefit rate of 7.65% is requested to cover FICA.

D. Travel
The budget accounts for two trips to Ithaca, New York for the PI to meet with Animal Trial Director. The trip is 640 miles round trip at $0.54 per mile = $364. Added to the cost of driving is a per diem of $126 per day for two trips. $490 x 2 = $980

F. Other Direct Costs
Materials and Supplies

Assembled circuit boards $4,000 x 3 runs = $16,000
Springs for flexible leads $2,000 x 2 batches = $4,000
Optical fiber $2,000 x 2 orders = $4,000
LED die chip minimum orders $1,000 x 3 = $3,000
Eutectic mounting of LED die chips $3,000 x 3 = $9,000
Encapsulation tooling $3,500

H. Indirect Costs
Rent of office space is $1,800 per month. This project would be a third of the effort of the organization so rent for this project would be $600 per month for the 9 months of the project.

General Supplies include: paper, ink cartridges, syringes for manufacture, epoxy for coating, silicone, solder iron tips, and other items used daily for design and manufacture in our facility.

Accountant and annual tax audit costs $12,000. A third of the audit will be accounting for funds from this grant, if awarded.

Operational office fees such as Nitrogen tanks and Internet at $60 per month. This project would be a third of the effort of the organization so $20 per month for 9 months of the project.

Insurance annually is $1,500 for Open Source Instruments. This project would be a third of the organization's effort so the charge to this project would be $500.

J. Fee
A fee to Open Source Instruments of not more than 7% of combined direct and in-direct costs is requested. $8,000

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		119,530.00
Section B, Other Personnel		6,459.00
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		125,989.00
Section C, Equipment		0.00
Section D, Travel		980.00
1. Domestic	980.00	
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		99,384.00
1. Materials and Supplies	39,500.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	59,884.00	
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)		226,353.00
Section H, Indirect Costs		10,080.00
Section I, Total Direct and Indirect Costs (G + H)		236,433.00
Section J, Fee		8,000.00
Section K, Total Costs and Fee (I + J)		244,433.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: 8726124450000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Cornell University

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Chris		Schaffer		Neuroscientist	142,000.00		0.09		1,422.00	500.00	1,922.00

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person **1,922.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6.0			17,816.00	0.00	17,816.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**17,816.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**19,738.00**

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

Funding Opportunity Number: PA-18-871 . Received Date: 2018-09-05T11:19:48.000-04:00

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

ORGANIZATIONAL DUNS*: 8726124450000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Cornell University

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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)

Tracking Number: GRANT12704511 Funding Opportunity Number: PA-18-871 . Received Date: 2018-09-05T11:19:48.000-04:00

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

ORGANIZATIONAL DUNS*: 8726124450000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Cornell University

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	5,630.00
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	
8 . Animal Costs	5,700.00
9 . GRA Tuition and Mandatory Health Fee	8,932.00
Total Other Direct Costs	**20,262.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**40,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Facilities & Administrative Costs - Endowed Research	64.0	40,000.00	19,884.00
		Total Indirect Costs	**19,884.00**

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)	**59,884.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	59,884.00

L. Budget Justification*	File Name: Budget_Justification_Cornell.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification – Cornell University

A. Senior/Key Person

Subcontract Principal Investigator, Prof. Chris Schaffer, Ph.D. (9-month appt.): This proposal requests salary support for 0.09 month academic year effort each budget year for Dr. Chris Schaffer. Dr. Schaffer will oversee the subcontract work. He will also guide the graduate research assistant in the planning and execution of experiments, in the analysis of data, and in the preparation of manuscripts and conference presentations for dissemination of the work.

B. Other Personnel

Graduate Research Assistant, To Be Named (12-month appt.): This proposal requests salary support for 6 calendar months of effort for a basic GRA appointment for 1 graduate students in each budget year. The salary support includes stipend each period. GRA total support is calculated at the NIH cap. The GRA will complete the research as outlined in the Cornell statement of work under the supervision of Dr. Schaffer.

All Cornell University non-student salaries are budgeted with an increase in July of each budget period in accordance with Cornell University policy with the exception of personnel with salaries above the current NIH salary cap. Salaries are based on current FY 18/19.

Consistent with federal cost principles, Cornell University estimates personnel time on a percentage of total effort. Cornell University does not track work hours for FLSA (Fair Labor Standard Act) exempt staff, and is unable to provide billing or time records based on hours. In accordance with OMB 2 CFR Part 200 §430(i), Cornell allocates a level of effort utilizing a Plan Confirmation System. The percentage of effort has been converted into months of effort.

Employee Benefits

Employee Benefits have been proposed at a rate of 34.9% for all non-student compensation through June 30, 2019, 35.3% from July 1, 2019 through June 30, 2020 and 35.7% effective July 1, 2020 for Cornell's endowed colleges. These rates are approved by the Department of Health and Human Services. See *https://www.dfa.cornell.edu/capitalassets/cost/employee* for more information about Employee benefit rates.

Materials and Supplies

Funds are requested in each budget year for purchase of materials and supplies necessary to complete the proposed project. These include, but are not limited to, disposable surgical supplies and drugs for inducing seizures and implanting the EEG/optogenetic devices in mice as well as miscellaneous laboratory needs including personal protective equipment and general use reagents and labware.

Other Direct Costs

Graduate Research Assistant, To Be Named (12-month appt.): This proposal requests support for 6 calendar months of effort for a basic GRA appointment for 1 graduate students in each budget year. The support includes tuition and mandatory health insurance fees each period. GRA total support is calculated at the NIH cap. The GRA will complete the research as outlined in the Cornell statement of work under the supervision of Dr. Schaffer.

Animal Costs: Funds are requested for animal costs necessary to complete the proposed research. These include, but are not limited to, the purchase, breeding, genotyping, housing, and veterinary care of the mice needed for the proposed research. Housing costs are $0.72/cage/day. Cornell staff bill hourly for husbandry services. Genotyping is conducted by an external contractor.

Facilities and Administrative Costs (F&A)

F&A costs have been proposed at a rate of 64% for Endowed Research effective July 1, 2018. The rate is approved by the Department of Health and Human Services. See *http://www.dfa.cornell.edu/sites/default/files/dhhsrateagreement.pdf.*
Modified Total Direct Cost exclusions include Capital Equipment, GRA Tuition and Health Fees, and Subcontract costs in excess of $25,000 per subcontract.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		1,922.00
Section B, Other Personnel		17,816.00
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		19,738.00
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		20,262.00
1. Materials and Supplies	5,630.00	
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	5,700.00	
9. Other 2	8,932.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		40,000.00
Section H, Indirect Costs		19,884.00
Section I, Total Direct and Indirect Costs (G + H)		59,884.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		59,884.00

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.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	206,469	0	0	0	0	**206,469**

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

Agency to which you are applying (select only one)*

❍ DOE ❍ HHS ❍ USDA ● Other: NIH

SBC Control ID:* 000814955

Program Type (select only one)*

● SBIR ❍ STTR

❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

Application Type (select only one)*

● Phase I ❍ Phase II ❍ Fast-Track ❍ Direct Phase II ❍ Phase IIA ❍ Phase IIB

❍ Commercialization Readiness Program (See agency-specific instructions to determine application type participation.)

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 4

1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?* ❍ Yes ● No

1d. Is your small business a Faculty or Student-Owned entity?* ❍ Yes ● No

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ❍ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and email address of the official signing for the applicant organization to state-level economic development organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ❍ No

7.Commercialization Plan: The following applications require a Commercialization Plan: Phase I (DOE only), Phase II (all agencies), Phase I/II Fast-Track (all agencies). Include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ❍ Yes ● No

Attach File:*

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ● Yes ❍ No

STTR-Specific Questions:

Questions 10 - 12 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 - 12 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ❍ Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ❍ Yes ❍ No

12. Provide DUNS Number of non-profit research partner for STTR.*

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OMB Number: 0925-0001

Expiration Date: 03/31/2020

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)

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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. 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

5. 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:

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Expiration Date: 03/31/2020

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3. Research Strategy*	Research-Strategy-20180905-0751.pdf
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Other Research Plan Section	
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6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
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Tracking Number: GRANT12704511
Funding Opportunity Number: PA-18-871. Received Date: 2018-09-05T11:19:48.000-04:00

Specific Aims: Optogenetics is a research field that utilizes light to activate transgenically or virally transduced photosensitive protein channels to excite or inhibit neurons[13,19,20,52,57]. Researchers can not only target specific regions of the brain through focused illumination but they can also genetically target specific groups of neurons within that region by utilizing promotor driven cell-specific expression of photosensitive channels. The high specificity offered by optogenetics creates many *in vivo* research opportunities to study psychiatric and neurological conditions. Conditions including epilepsy, Alzheimer's, OCD, and schizophrenia have characteristic abnormal EEG patterns[5,6,16,29,45,57]. To study these conditions and find treatments, researchers utilize **mouse models to genetically and spatially target neuron groups for activation and inhibition while monitoring electrophysiology in one or more brain regions**. At the moment, these experiments are very difficult to perform due to the lack of a device that can perform wireless EEG monitoring and optical stimulation in mice. While tethered equipment has been sufficient to show the promise of optogenetics, it has severe practical shortcomings including short testing times and prohibiting cohabition which limit experiment design[32]. Therefore, **neuroscientists in several research areas have asked us for a wireless instrument capable of EEG recording and optical stimulation in mice.** We propose an instrument that not only delivers this capability, but that also paves the way for **optogenetics as a functional neurosurgical intervention**. The instrument will be able to detect aberrant EEG patterns and then deliver optogenetic stimulation per closed-loop feedback. This will allow our customers to deliver functional intervention in neural circuits exhibiting a disease state while having little or no impact on healthy brain function. We will test the intervention hypothesis on a mouse model of focal epilepsy. This will advance our long-term goal of creating **a fully implantable medical device that detects the onset of seizures and aborts them with optical stimulation** akin to how implantable defibrillators detect and abort arrhythmias.

Aim 1: Build a fully implantable EEG monitor with optical stimulator that is suitable for use with transgenic mice. It will be called the Mouse-Sized Implantable Stimulator with Lamp (MS-ISL). OSI has already developed each of the core building blocks necessary for the instrument. Our main product is a fully implantable instrument that transmits high-fidelity EEG data in mice or rats for over four months at a time[26]. We've also developed and tested an optogenetic stimulator with EEG recording that was proven capable of inducing optogenetic effects in rats. This 4.2 mL product was built as a proof-of-concept; It has very limited research utility on its own due to the difficulty of designing experiments for rats compared to mice. To access the enormous pool of transgenic mouse strains that are validated as disease models and that are sensitive to optogenetic stimulation, our customers request that we provide an optogenetic stimulation device compatible with mice. We will design an instrument that has a volume of 1.5 mL that is capable of recording high-fidelity EEG and applying optogenetic stimulation upon receipt of wireless, individually addressed commands. This will give researchers access to the diverse pool of transgenic mice.

Aim 2: Embed OSI's proven PC-based EEG event detection software in a 2.5 x 2.5 mm logic chip, enabling completely autonomous, closed-loop response. The MS-ISL will wirelessly transmit EEG data to our existing PC-based data acquisition system in real-time. The PC-based software detects events and commands optical stimulation[60,61]. To move toward a self-contained medical device, we will embed our computationally efficient detection algorithm directly in the MS-ISL itself, permitting it to be used without the PC. We will take 200 hours of archival EEG data recorded by our customers and play it into the logic chip. We will show that it can calculate each classification metric and autonomously classify events including seizures, ictal pulses, and post-ictal depression in real-time. It can respond with stimulation or simply report the events.

Aim 3: Demonstrate the MS-ISL's ability to detect seizures and interfere with them by applying closed-loop optogenetic stimulation. Our goal is to test the utility of the MS-ISL for monitoring brain activity, identifying aberrant activity patterns, and modulating the firing of genetically-targeted neurons through optogenetic stimulation. We will demonstrate this in a mouse model of focal epilepsy, which provides an ideal testbed because the appropriate site for electrophysiological recording and optogenetic activation is clear. The subcutaneously-implanted, wireless MS-ISL will monitor cortical EEG at an epileptic focus, identify seizure initiation, and turn on the fiber-coupled LED to optogenetically stimulate neurons to interrupt the seizure. We will test this scheme both by exciting inhibitory interneurons using Channelrhodopsin-2 (Ch2) in one group of transgenic animals and by inhibiting excitatory pyramidal neurons using Halorhodopsin (HR) in another. We will induce chronic focal neocortical epilepsy by intracranial nanoinjection of iron chloride, which simulates the conditions of traumatic focal epilepsy induction and leads to the development of seizures over time. The MS-ISL will be implanted and left off for about two weeks until the epilepsy develops so that the mice are having about 25 seizures an hour. We will then begin recording sessions with half the time allocated to just recording brain activity and the other half with the optogenetic feedback turned on. We will demonstrate the effect of the MS-ISL's optogenetic feedback in a 14-week, chronic experiment. Our primary assay will be a statistical reduction in the duration of seizures when closed-loop optogenetic stimulation is applied.

1. Significance

We propose to build a fully implantable EEG monitor combined with an optogenetic stimulator. The instrument will both: **1.** Fulfill an immediate commercial need that advances basic neuroscience and disease research; and **2.** Advance our long-term objective of creating a medical device that uses closed-loop optogenetic response as functional intervention to treat disease states such as focal seizures.

1.1 An instrument that advances our understanding of the brain and human disease

In the last decade, optogenetics has grown from being the subject of just a dozen papers in 2009 to over 950 in 2017[38]. It evolved from being an experimental technique for activating neurons *in vitro* to being a means of studying neuropsychiatric diseases *in vivo* with mouse models of conditions including schizophrenia, epilepsy, and Parkinson's[13,19,20,52,57]. The promise of the technology comes from its ability to target highly specific neuron groups both spatially, temporally, and genetically. For example, researchers can design an experiment in which a mouse model expresses Halorhodopsin (HR) in cortical pyramidal neurons, and they can subsequently activate those neurons with pulses of light [21,43]. Many neuroscientists want to apply optogenetics to their own research topics. However, they are often unable to proceed due to a lack of suitable equipment. Instrumentation companies have failed to keep pace with the progress and imagination of neuroscientists. This has created a market opportunity.

Commercially available hardware typically requires that animals are tethered by their heads to benchtop equipment. This places severe constraints on experiment design, and adds a cost to obtaining usable data that is often prohibitive. For example, *i)* regulations limit the amount of time that a tether can be used each day; *ii)* tethers require frequent human intervention throughout the course of the experiment; *iii)* tethers are incompatible with experiment methodologies such as operant chambers and mazes; *iv)* animals cannot cohabit; *v)* animals cannot engage in natural behavior during recording (socialization, moving into enclosed spaces, using exercise wheels, etc); *vi)* externally protruding hardware is prone to animal self-mutilation[32]; and *vii)* the physical tether often introduces movement artifacts in data that increase the cost of data analysis. To make optogenetics more accessible, several groups have developed wireless optogenetic stimulators. However, they are all **fundamentally incompatible with electrophysiological recording**, limiting their use to behavioral experiments.

Consider the class of conditions in which abnormal, characteristic EEG patterns are present. These include epilepsy, Alzheimer's, OCD, and schizophrenia[5,6,16,29,45,57]. To study these conditions and their cures, researchers would like to **simultaneously monitor EEG and apply optogenetic stimulation**. For these experiments to be practical, researchers need a wireless, fully implantable instrument capable of both EEG recording and optical stimulation. Open Source Instruments Inc (OSI) is capable of designing this instrument and subsequently making it available for sale alongside its existing EEG telemetry products. We will call the instrument the Mouse-Sized Implantable Stimulator with Lamp (MS-ISL).

> "I confirm that my laboratory would be keen to purchase the proposed Mouse-Sized Implantable Sensor with Lamp (MS-ISL) for implantation in mice. … The proposed development work represents important steps both towards clinical translation and towards testing circuit theories of brain function. I foresee many additional applications by our group and others." - *Dr. Dimitri Kullmann, Professor of Neurology, University College London (U.K.)*
>
> "Therefore, I confirm that my lab would use the proposed mouse-sized Implantable Stimulator and Monitors with fiber-coupled LEDs. The development of the new technologies described by Kevan Hashemi in this proposal is, in my opinion, a fundamental step towards making discoveries that contribute to ameliorate the burden of mental health disease through the world." - *Dr. Analisa Scimemi, Assistant Professor, University at Albany (New York)*

1.2 A medical device that uses optogenetics as functional neurosurgical intervention

The MS-ISL will not only create the basic research opportunities described above, but will be immediately used as a means of functional intervention to treat disease states in the brain. Our long term objective is to provide medical devices that monitor EEG activity and automatically respond to aberrant events by applying correcting pulses of optogenetic stimulation. The specificity of stimulation means that disease states can be treated with minimal impact on healthy brain function[3,4,44,51]. While many disorders may be treatable with this approach, we will start with focal seizures for the reasons described below.

Several groups, including one of our customers, have shown that seizures can be halted or reduced by the optogenetic activation of inhibitory neurons with the use of Chennelrhodopsin-2 (Ch2)[12,28,43,55,60]. It has also been shown that expressing Halorhodopsin (HR) in cortical pyramidal neurons can reduce seizure propagation[28,31]. We will test the ability of the MS-ISL to interfere with seizures via both the Ch2 and HR approaches at the Schaffer Lab at Cornell.

Focal seizures initiate in one location and propagate out to other regions in the brain. This means that it is obvious which region of the brain we must target for both EEG monitoring and optical stimulation. Furthermore, we have analyzed the EEG signals of focal seizures and have already demonstrated the ability of our PC-based software to automatically identify them in real time[24,60].

We will work with focal epilepsy models not just because it is an ideal testbed for the technology, but because it could have a direct impact on human health. Partial or localization-related epilepsy is the most common seizure disorder, affecting approximately one half of the 50 - 60 million people who suffer from epilepsy[7,17,48]. Approximately 45% of these patients experience medically untreatable focal seizures that

are physically and socially disabling[15]. In the future, we could evolve the MS-ISL into a a **fully implantable medical device for humans that detects the onset of seizures and aborts them with optical stimulation** akin to how implantable defibrillators detect and abort arrhythmias.

2. Innovation

2.1 Biometric Instruments at Open Source Instruments, Inc.

Open Source Instruments Inc. (OSI) was founded in 2004 to design equipment for scientific research[35]. OSI appeals to scientific customers by providing complete characterization of all of its instruments and software.

Soon after its founding, OSI entered a collaboration with Dr. Matthew Walker, Institute of Neurology (ION), University College London (UCL), to develop an implantable, wireless EEG monitor for epilepsy studies in rats. After five years of collaboration, OSI demonstrated an effective and reliable wireless EEG monitor[9,41]. OSI's fully implantable monitor produces recordings free of noise and artifact, which allows us to automatically detect EEG events such as seizures, ictal spikes, inter-ictal spikes, etc in our PC software. OSI telemetry products and software are now used in both mice and rats [6,9,10,11,18,24,26,30,50,51,59,60]. Our telemetry products have been profitable since 2009.

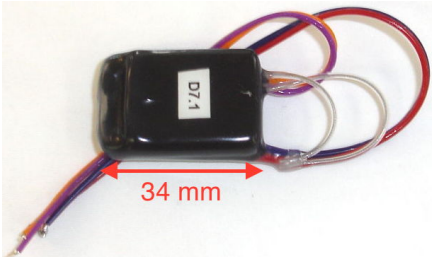

Figure 1a: OSI's Implantable Sensor with Lamp (ISL) is a proof-of-concept used to record EEG signals and drive optogenetic stimulus in rats. This component is implanted in the abdomen where it doesn't interfere with animal behavior. Electronics and battery are enclosed in the black epoxy package. The silver loops are antennas for receiving commands and transmitting live data. The colored leads record EEG signals and drive the LED.

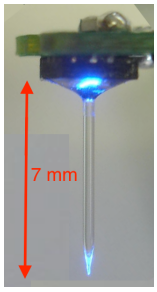

Figure 1b: The Fiber Coupled LED (FCL) is the satellite head fixture driven by the ISL. It is mounted to a hole in the skull with dental cement. Blue light is visible at the LED surface and at the coupled fiber's tapered tip.

OSI's experience with EEG monitors and automatic seizure detection gives it two of the three core technologies necessary to build an optgoenetic stimulator with closed-loop response. The final ingredient is the ability to inject light into neural tissue. To develop a proof-of-concept, OSI added an optogenetic stimulus capability for its rat-sized EEG monitor. We developed this device in collaboration with Dr. Dimitri Kullmann (ION, UCL) and called it the Implantable Sensor with Lamp (ISL) [42] [Figure 1]. **The ISL is a proof of concept. It is a wireless, subcutaneous device implanted in a rat's abdomen. It contains a battery, antennas, and the electronics required for command reception, EEG recording, live EEG data transmission, and driving an LED for optogenetic stimulation.** The device does not use an external tether during any stage of deployment and there are no external components that can be scratched or chewed.

Subcutaneous leads connect the ISL to a satellite head fixture that we call the Fiber-Coupled LED (FCL). The FCL houses an LED coupled to an optical fiber that is tapered to a sharp point that minimizes the formation of scar tissue.

The ISL has been implanted in rats and is proven to provoke an optogenetic response (behavioral changes and induction of seizure)[34]. Preliminary results indicate its ability to abort seizures [61]. While the ISL demonstrated the principle of an optogenetic medical device, its research utility is actually severely limited by the lack of transgenic rats that express opsins or which are validated as disease models. The proof-of-concept ISL cannot classify EEG events on its own, but relies upon processing in PC software. It is also a disposable device, usable for only a single experiment. The proposed MS-ISL will be re-chargeable and will be capable of autonomous, closed-loop EEG event classification.

2.2 The Need for a Mouse-sized Device

When rats are used in optogenetic experiments, a viral vector must be used to express the photosensitive protein that controls ion channels. This method is unreliable, imprecise, and requires expertise not available to many laboratories. By contrast, a wide variety of transgenic mouse strains are readily available which express opsins in specific neuron subsets. Furthermore, many mouse strains are available as validated models of human disease. **Mouse models offer far more opportunities for optogenetic experimentation than rats**. To be commercially viable and have a significant impact on neuroscience, we must take the technologies we demonstrated in the ISL, and implement them in a device that is compatible with mice. This is not an iteration, but **a redesign that will result in a**

"[The proposed project] would allow us to make use of the wide range of genetically modified mouse strains that allow optogenetic actuators (opsins) to be expressed selectively in different populations of neurons (either excitatory or inhibitory). There are also many strains that have been validated as models of epilepsy, schizophrenia and other neurological disorders, further underlining the need to perform these experiments in mice." - *Dr. Dimitri Kullmann, Professor of Neurology, University College London*

"Most optogenetics projects are conducted in mice (rather than rats) given the exclusive availability of a large variety of transgenic mice allowing the targeting of many specific cell types. So, an optogenetic stimulator suitable for implantation in mice is of great value to the wider research community!" - *Dr. Dennis Kätzel, University of Ulm*

commercial product that has 1.5 mL volume compared to the proof-of-concept ISL's 4.2 mL volume, thus enabling a whole new class of experiments.

2.3 Closed-loop Autonomous Response

A major barrier to functional intervention devices is the ability to reliably detect subtle EEG abnormalities with a tiny, low-power device that may have to run for a decade on a single battery. The MS-ISL will the **the first implantable device in the world that is capable of autonomously classifying EEG events** to determine when intervention is necessary. We will use the computationally-efficient event classification algorithm that OSI developed and proved successful in its PC-based software, ECP19 [24,60]. We will implement the algorithm in a 2.5 x 2.5 mm logic chip optimized for EEG event classification. It will detect events such as seizures, ictal pulses, inter-ictal spikes, polyspikes, and post-ictal depression. The MS-ISL can be used *either* with the the PC-based ECP19 forming a part of the closed-loop *or* completely autonomously, with processing done onboard the MS-ISL.

2.4 A Focal Seizure Model to Test the MS-ISL

To demonstrate the MS-ISL's utility as an intervention device, we will use a recently developed model of traumatic injury and cerebral microhemorrhage using a nanojnjection of iron chloride. Cerebral microhemorrhages are common in both traumatic brain injuries and aging brains, especially ones with degenerative diseases. These small bleeds can lead to increased incidences of inflammation as well as an increase in the loss of the contents of plasma into the brain tissue. The increase in certain compounds like hemoglobin and iron within the brain tissue creates an accumulation of oxygen and reactive oxygen species (ROS). The increase in iron attracts more oxygen and ROS to the micohemorrhage and as well as the increase in extracellular plasma brings more glutamate to the site causing excitotoxicity. The increase in ROS can cause neural rewiring that induces a focus for seizure activity[49,56]. In order to simulate the environmental factors that occur during traumatic focal epilepsy induction, an iron injection is used to simulate the damaging effects of a microhemorrhage that lead to the creation of an epileptic focus[23,58]. This model has shown 70% viability to mice and shows that about 25 seizures an hour with a length of about 4 seconds per event[23]. This model is an ideal testbed for the MS-ISL, as it is clear that we would want to monitor EEG and modulate activity at the epileptic focus, and the high rate of events will provide ample data.

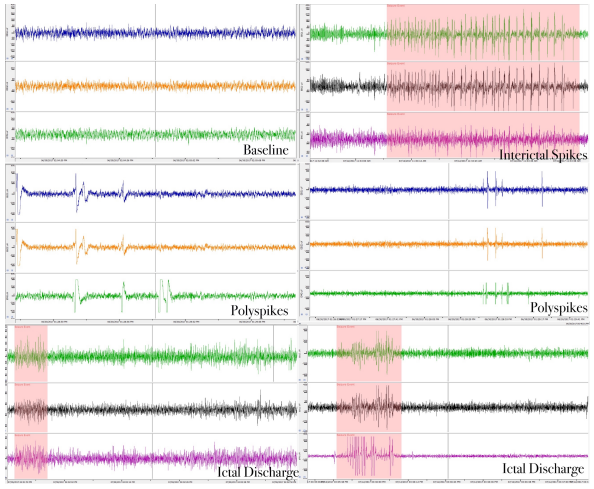

Figure 2. Nanoliters of iron chloride injected into the cortex leads to chronic focal neocortical epilepsy. These are EEG recordings from three implanted electrodes on the mouse's motor cortex. Seizure focus is induced at the placement of the second electrode by nanoinjection of iron chloride. Seizures then propagate to electrodes three (~1 mm away) and then finally electrode one (~2 mm away). Box shows multiple recordings of characteristic epileptiforms from local field potentials including polyspikes, ictal discharge, and interictal spikes.

3. Approach

3.1 OSI's approach compared to existing technology

Several companies provide optogenetics technology which at first appears suitable for transgenic mouse experiments. To overcome the shortfalls of tethered equipment, groups have developed wireless instruments that can deliver optogenetic stimulation to mice [21,25,32,53]. The existing products are compared to our proposed instrument in Table 1. None of them are adequate for experiments requiring biopotential monitoring, as described below.

None of the competing devices are compatible with electrophysiology recording. Our proposed instrument will be capable of EEG recording with performance very similar to that of our commercially available monitors (Figure 3). **Key specifications:** *i)* records at 512 samples per second with performance optimized for signals between 0 and 160 Hz; *ii)* total noise is 8 µV root mean square (rms); *iii)* 20 mV dynamic range; *iv)* no artifacts due to instrument movement. For reference, baseline EEG levels are around 50µV rms and may exceed 1000µV rms during seizures (SNR ranges between 5 and 100). The digitized signal is transmitted using ultra low power 915 MHz telemetry which has no observable effect on the EEG measurement.

Some wireless optogenetic equipment relies on wireless power transfer to run the instrument because their electronics are not efficient enough to be powered by a battery[25,27,32]. The contact between brain tissue and an EEG electrode demodulates the wireless power oscillations and produces low-frequency artifacts in the EEG recording. **Wireless power systems are certain to irrevocably corrupt EEG signals in all cases.**

We believe that the only way to make a practical implantable optogenetic stimulator with monitoring capability is to provide power with a battery, as proven effective by our available products. Unlike other devices which rely on battery power, our circuits are ultra-efficient and capable of running an entire experiment without being recharged. **Our proposed device will be capable of 160 days of standby time, or over one week of continuous data recording and periodic optical stimulation [Table 2].** This compares to a standby time of less than 1 day for competitors' devices [Table 1]. The device will be surgically implanted in standby mode. Once the model has fully recovered and researchers are ready to begin collecting data, they send a command to switch the instrument on; it is only then that the device begins consuming significant power. Experiments which do not require continuous EEG monitoring can set the device to standby mode when not in use. For example, **if an experiment requires 4 hours of EEG monitoring per day, the device will run for 32 days**.

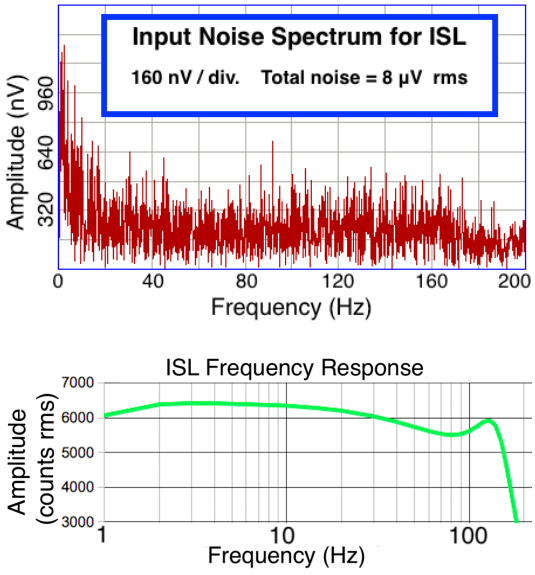

Figure 3 - Biopotential performance of the ISL The proposed instrument will have EEG recording characteristics very similar to the ISL. **Top:** Input noise spectrum for the ISL. **Bottom:** Frequency response of the ISL. A three pole filter is used to limit the contribution of signals above 160 Hz.

Another major advantage of our proposed device compared to others is the physical packaging. All of the mouse-sized optogenetic stimulators for sale today are mounted external to the skull of the animal, presenting several issues: *i)* external systems weigh approximately the same as the mouse's head, making them unwearable for continuous use, *ii)* the large, externally protruding device inhibits normal behavior and social interactions with other mice, and *iii)* mice will injure themselves and each other by scratching and chewing on external devices[32]. Our instrument design avoids these shortcomings by using a fully implantable, two-part design [Figure 1]. **The benefits include animal cohabitation and improved welfare; the ability to record data 24 hours per day; the elimination of external components prone to scratching or chewing; and improved reliability.**

Our proposed instrument will be much more practical than any other on the market. **Once implanted, the device will require absolutely no physical interaction by the researcher.** It is completely controlled by wireless commands, and will easily endure over 100 days of implantation per charge.

Table 1: Survey of Current Wireless Optogenetic Devices

	OSI MS-ISL	Kendall [25]	Teleopto [53]	Riken [21]	Poon Lab [32]	NeuroLux [33]
EEG monitoring	Yes	No	No	No	No	No
Location	subcutaneous	head mounted	head mounted	head mounted	subcutaneous	subcutaneous
Volume	1.5mL	~3mL	1.6mL	~2mL	0.1 mL	0.1 mL
Power Source	Battery	RF	Battery	Battery	RF	RF
Standby Time	3800 hours	NA	17 hours	20 hours	Indefinite	Indefinite
Programmable	Yes	Yes	No	No	Yes	Yes
Individual Control	Yes	Yes	Yes	Yes	No	No
For Sale	Planned	No Longer	Yes	No	No	Yes
Can target deep brain	Yes	?	Yes	Yes	Yes	No
Consistent stimulus	Yes	?	Yes	Yes	No	No

3.2 Aim 1: Build a fully implantable EEG monitor with optical stimulator

We will use the existing ISL circuit as a starting point for the MS-ISL design. We will substitute all of the components in the current design with similar components in smaller packages. For example, the logic chip is 16 mm on each side, but can be substituted with a chip that is functionally identical, but only 2.5 mm on each side. We will further reduce the volume by designing a single dual purpose antenna that replaces the two antennas in our proof-of-concept, ISL. The total volume of the circuit board and battery will be 1.5 mL once assembled and coated.

Our proof-of-concept ISL is a disposable device designed for a single experiment. For the commercial MS-ISL proposed here, we will design recharging circuitry that allows the instrument to be reused. Researchers can return the MS-ISL after explantation for refurbishment and re-certification. **SBIR Phase I development will allow us to profitably sell the MS-ISL for $700 new and $350 refurbished**

(including a new optical head fixture). The **fixed cost of all bench top equipment required to use the MS-ISL is $12,500 and supports up to 30 cohabiting mice**. The bench top equipment is already available for sale per our EEG telemetry product line.

One of the largest barriers to creating a mouse-sized optogenetic instrument is the challenge of delivering sufficient optical power to activate opsins without rapidly depleting the battery. Gathering light from an LED surface and injecting it into an optical fiber is inefficient when using commercially available LEDs and fibers. We have tested solutions to this problem by *1)* having custom optical fiber manufactured with a refractive index of 1.63; *2)* mounting the fiber to a custom wire-bonded bare LED die, and *3)* building a machine that tapers the optical fiber tip to maximize radiant flux and minimize the formation of opaque scar tissue. To fit the optical component into a mouse-sized device, we will use the Cree TR2227 LED die. Since this chip is just 240 x 320 µm across, we will need special procedures to handle it. We have consulted with a reliable supplier who is confident in their ability to mount TR2227 dies for us using their equipment. The TR2227 will be mounted on our substrate with a eutectic gold bond.

Table 2: Calculated runtime for the MS-ISL per charge

Operation Mode	Current draw	Runtime
Standby Mode	5 µA	> 158 days
Optical stimulation for 30 minutes per day	28 µA	30 days
Epilepsy Experiment	25 µA	32 days

Runtime for the proposed device is estimated based on current consumption measurements taken in our lab.
Standby mode: the device is inactive but able to receive commands and start recording or optical stimulation
Optical Stimulation 30 minutes per day: the lamp is switched on for 2 ms pulses at 10 Hz repetition with 9 mW optical power at the fiber tip. This intensity and duty cycle has been shown to induce behavioral changes [61].
Epilepsy Experiment: Uses 4 hours of EEG recording per day and optogenetic stimulation in response to each of 25 seizures per hour (10 s stimulation per seizure)

We will re-tool our machine to craft 220 µm diameter fiber tapers. Since all units are built to order, optical fiber length may be specified by the customer. Customers who only need to illuminate the surface of the brain rather than targeting a deep region will be able to order the instrument without a coupled fiber and simply rely on the LED illuminating brain tissue through a section of thinned skull.

The MS-ISL will produce at least 4 mW of 460 nm light from its fiber tip at its default operating current of 40 mA. Even 2 mW is sufficient to activate channelrhodopsin-2 (Ch2) and halorhodopsin (HR) molecules in mammalian neurons [4]. These custom procedures will allow us to **deliver far more optical power into deep neural tissue than any other company for the same amount of electrical energy expended**. Customers can modulate the optical power by selecting different LED duty cycles. Customers can order either a blue version (~460 nm) for use with Ch2 or green version (~530 nm) for use with HR.

3.3 Aim 2: Embed OSI's proven PC-based EEG event detection software in a 2.5 x 2.5 mm logic chip, enabling completely autonomous, closed-loop response.

OSI provides Event Classification Processor software (ECP19) to automatically classify EEG events. ECP19 calculates 7 metrics on EEG data including power, coastline, intermittency, coherence, asymmetry, and spikiness. Using recordings that our customers have made with our instruments, we have developed an Event Library that includes baseline, ictal spikes, inter-ictal spikes, polyspikes, and various kinds of seizures. ECP19 automatically classifies data as belonging to the appropriate event. In addition to using the events defined by OSI, the software allows our customers to develop their own event definitions. ECP methodology was published in [60] and it has been used to replace or augment human classification in several published papers since then. ECP19 runs on a PC (Linux/Mac/Microsoft). It can be used in real-time or after an experiment to classify thousands of hours of data.

ECP19 can be used with the MS-ISL to provide closed-loop feedback. ECP19 will process MS-ISL EEG data in real time, and ECP19 will wirelessly command optogenetic stimulation in response to appropriate events. This has already been tested using the proof-of-concept device [61].

In order to provide functional intervention, a medical device will have to perform event classification autonomously. The challenge is that the processing could run down the device's battery quickly and make the device impractical. This is especially true as the device must be capable of distinguishing between subtle differences in EEG events. Fortunately, the algorithm underlying our ECP19 software is fundamentally computationally efficient. We will use the MS-ISL to prove that autonomous processing is practical.

We will build a microprocessor in the same programmable logic chip that handles EEG recording, telemetry, and command reception (LCMXO2-1200ZE). This chip is highly energy efficient. The microprocessor will be programmed in a custom version of Z80 assembler. It will be optimized for the fundamental mathematical operations that underly our calculation of EEG metrics. We can then program the MS-ISL to trigger on any particular event type that can be classified according to the underlying metrics. We will test the device in our lab by feeding it at least 200 hours of data recorded from at least

four independent customers. We will compare its ability to calculate EEG metrics to the capability of the PC-based ECP19.

For the *in vivo* test in Aim 3, the PC-version of ECP19 will command optical stimulation. Independent of the closed-loop desktop processing, the MS-ISL will use telemetry to report when it independently detects seizures. At the end of the experiment, we will compare the success of the MS-ISL at detecting seizures to the PC software.

3.4 Aim 3: Test the MS-ISL *in vivo.* We will interfere with focal seizures using the implantable EEG recording and optogenetic stimulation device.

Seizures are an abnormal increase in excitatory activity within the brain. Focal seizures initiate in one location and propagate out to other regions in the brain. These seizures can't be medically managed in 45% of human patients[7]. It has been previously shown that seizures have the ability to be halted or reduced by optogenetic activation of inhibitory neurons with the use of Channelrhodopsin-2[12,28,43,55,60]. It has also been shown that expressing Halorhodopsin (HR) in cortical pyramidal neurons can also reduce seizure propagation[28,31]. Therefore, we propose to utilize two different types of transgenic mice: one expressing Ch2 in cortical interneurons and one expressing HR in pyramidal cortical neurons. ChR2(H134R)-EGFP transgenic mice from Jackson Labs have a Cre-inducible Channelrhodopsin-2 (Ch2) knock-in and will be crossed with animals expressing a transgene for the tamoxifen-inducible Cre in cortical interneurons. This creates mice that have Ch2 expressed in cortical interneurons, so that when blue light (~460 nm) illuminates the tissue it will cause a conformational change in the channel allowing cations in to depolarize the membrane[2,19,54,62]. This activation of inhibitory neurons can directly inhibit cortical pyramidal neurons during seizures to reduce the excessive synchronous activation of the brain. Another subset of transgenic mice, Thy1-eNpHR2.0-EYFP, will be used in a similar fashion to express HR in cortical pyramidal neurons. These neurons when illuminated will induce a conformational change in a chloride channel causing the neurons to hyperpolarize and can reduce seizure activity[20]. The use of these transgenic mice in combination with the wireless MS-ISL will allow for to simultaneous monitoring of EEG activity in the brain and optogenetic activation of interneurons or inhibition of cortical pyramidal neurons through the FCL during seizure propagation. We will evaluate whether closed-loop stimulation shortens seizure length.

Methods: We have constructed a paradigm to investigate the ability of the MS-ISL to monitor brain activity over time while optogentically minimizing seizure propagation. First, we will create a burr hole in the skull of our transgenic mice to induce chronic focal neocortical epilepsy and introduce the MS-ISL. We will utilize a recently-published model of focal epilepsy in rodents that relies on the microinjection of an iron chloride solution into the cortex[23]. Briefly, we will inject 350 nL of 100 mM $FeCl_3$ at the center of the burr hole at a depth of ~500 μm beneath the cortical surface. In preliminary data, we have found that all iron-injected animals showed epileptic activity within a week or two, including polyspikes, interictal spikes, and full ictal discharges (Figure 2). We observed ~25 seizures per hour with this model. Immediately after iron chloride injection the FCL and EEG recording electrode will be inserted into the cortex at the injection site, and the MS-ISL will be subcutaneously implanted. Previous recordings from the Schaffer lab will be used to determine parameters for seizure detection by the MS-ISL. During testing, the device will record activity only for half of the session, then during the other half of the session the device will record and optogenetically stimulate whenever the ILS recognizes seizure-like activity. The light will flash for 10 s at 10 Hz to elicit optogenetic stimulation. Each group will have the device turned off for the first two weeks to allow time for the epilepsy model to develop. In this proposed study, we will break animals up into two groups based on the transgenic model. One group will include mice with Ch2 gene expressed in cortical interneurons and the other will express the HR gene in cortical pyramidal neurons. Each group will utilize the surgery described above. Both groups will have weekly four-hour recording sessions for 14 weeks with five animals per group. These recordings sessions will determine the ability of the device to monitor brain activity, detect seizures, and optogenetically reduce seizure propagation over long periods of time. We will video record sessions to examine behavioral changes during seizures and optogenetic manipulation of brain activity. We will compare the frequency, amplitude, and duration of seizures, as well as behavioral correlates, with the optogenetic feedback on vs. off in both the Ch2 transgenic mice and HR transgenic mice.

Expected Outcomes: Chronic recording of animals with iron chloride induced focal neocortical epilepsy should correlate with previous data from the Schaffer lab and show frequent focal siezures. It is expected that the MS-ISL will monitor brain activity for 14 weeks per animal, thus capturing about 1,400 potential seizures. Optogenetic stimulation in half of these potential seizures should lead to a reduction in the frequency, amplitude, and duration of the seizures. Our primary assay will be the observation of a statistical shortening of seizure length in response to closed-loop optogenetic stimulation. Overall the wireless system should show its ability to monitor brain activity while simultaneously detecting seizures and optogenetically minimizing their activity.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

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
Does the proposed research involve human specimens and/or data	❍ Yes ● No
Other Requested information	

USE OF VERTEBRAL ANIMAL SUBJECTS AT CORNELL

Our studies of seizure propagation necessitate the use of laboratory animals. We take every effort to ensure safe and humane handling and treatment of our animals and extract the maximum amount of useful data from each animal we use.

Animals will be obtained commercially and housed at an animal care facility that is located at Weill Hall, down the hall from the Prof. Schaffer's laboratory. Animals used for chronic EEG recordings will be housed in the same animal facility.

The care and experimental manipulation of our animals has been reviewed and approved by the Institutional Animal Care and Use Committee at Cornell University. We now address the five points mentioned in the Instructions.

1. Proposed use of animals. Our subjects are 15-40 g, heterozygous ChR2(H134R)-EGFP transgenic mice on a C57BL/6 background mice (10 per year), and heterozygous Thy1-eNpHR2.0-EYFP transgenic mice on a C57BL/6 background mice (10 per year), based on a usage of about three experiments per week. Since our experiments involve propagation of complicated neural activity patterns, such as seizures, cell culture cannot be used.

Anesthesia

During the surgery all subjects will be maintained at sufficiently deep anesthetic levels to eliminate any response to a firm footpad pinch. We will use inhaled isofluorane for surgery.

Surgery

Chronic epilepsy induction with mouse sized implantable sensor with lamp

Glycopyrolate is delivered by subcutaneous injection (500µg per kg mouse) at the start of surgery. We further supplement with 5% (w/v) glucose in physiological saline (5 ml per kg mouse) every hour. Body temperature is measured and is maintained at 37.5°C with a heating blanket. Blood oxygen levels and heart rate are monitored with a pulse oximeter. Eyes are protected with a covering of veterinary eye ointment. In acute animals, blood pressure is monitored through a catheter in the femoral artery. All surgical areas are shaved and cleansed with Povidone-iodine followed by swabbing with 70% (v/v) alcohol. Bupivacaine is injected into the skin at each incision. A burr hole is drilled into the skull on the right side near lamda where chronic forcal epilepsy induction will occur through nanoinjection followed by introduction of and electrode and the fiber coupled LED. A glass pipet attached to a nanoinject will be inserted 500 µm into the motor cortex near the burr hole. Once the glass pipet has been at the correct depth in the cortex for 5 minutes an injection of 350 nL of either saline or iron chloride will be made into the mouse's brain. The glass pipet must wait in the brain for a minimum of half an hour to avoid backflow. Once the glass pipet is removed the fiber coupled LED and electrode will be lowered to the new epileptic focus. Then the subcutaneous implant will be placed in the abdomen and sutured closed with wires and fibers that run subcutaneously to the head where the fiber coupled LED will be mounted and cemented down with C&B Metabond.

Chronic post-operative care

Chronic animals will be housed one to a cage after chamber attachment. The animals are judged to be in distress if there is a loss of grooming or appetitive or if the animals exhibit a reluctance to rise and move. Analgesics will be administered post-operatively if the animal is in pain as diagnosed by cowering in a corner of the cage, sustained immobility, or not eating or drinking. We use Ketoprofen at a dosage of 0.1 - 0.5 mg per 100 g animal weight, administered sub-cutaneously, once following surgery and again 24 and 48 hours later. Post-operative maintenance will be carried out continuously until the animal is awake and can regulate its body temperature. Typically, this occurs within 1 hour after surgery. At this time the animal is transferred to the animal housing facility. For each subsequent imaging session, the animal is reanesthetized and imaged for as little time as possible, typically less than 4 hours.

Electrophysiology/Optogenetic Stimulation

In chronic animals, there will be an implanted electrode and monitored for 4 hours every week for 100 days. During half of the recording time each week the full device will be turned on which will detect seizure initiation and activate the fiber coupled LED to flash for 10 s at 10 Hz to elicit optogenetic stimulation.

Epilepsy models

In chronic animals, a microinjection of 350 nL of 100 mM $FeCl_3$ will be made at a depth of ~500 µm beneath the cortical surface. This model leads to interictal spikes and ictal events after about 1-2 weeks.

2. Justification of use of animals. Our studies will require the use of approximately 20 transgenic mice per year, based on an average usage of 3 per week. Because this work involves the evaluation of layer-specific seizure propagation as well as neural activity in response to peripheral stimuli, it is not possible to use cell culture or other non-*in vivo* methods to obtain the results. We have chosen mice as our model system because of the large body of knowledge on seizure propagation in mice as well as reliable epilepsy animal models in mice. The use of mice also builds on the experience of the co-P.I.s and other laboratory personnel with these animals.

We emphasize that every effort will be made to get as much useful data as possible out of each animal, as a means to minimize the total number of animals required. In all cases, enough experiments will be performed to ensure statistically-significant results are obtained.

3. Veterinary care of animals. Cornell University is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC effective date: June, 2015), and has a current PHS Animal Welfare Assurance (ID: coming from Cornell Engineering department of sponsored research, I have emailed and called themA3347-01). Animals used in the proposed research will be purchased commercially and housed at an on-site animal housing facility at Weill Hall. The animal housing facilities are located approximately 100 m from the P.I.'s laboratory, in the same building. The animal facility provides 24 hour/ 7 day animal care, including weekends and holidays, by a highly-trained staff of veterinarians and animal health technicians.

Cornell-wide support services include veterinary care and consultation as well as training classes and seminars, including hands-on training labs. Access to surgical suites and other appropriate core facilities is also provided.

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Consortium and Contractural Arrangement

This proposal is a collaborative effort between Open Source Instruments, Inc. and Cornell University's Schaffer Laboratory in the Meining School of Biomedical Engineering. Open Source Instruments will serve as the prime institution for the project, and Cornell University will serve as the sub-recipient.

Open Source Instruments will design and build an optogenetic brain implant with EEG monitoring and response for mice. This will be a research tool to be placed subcutaneously in animals and will be designed in order to support the search for effective therapeutic interventions for Epilepsy.

Prof. Schaffer's laboratory at Cornell will take the wireless EEG/optogenetic devices produced by Open Source Instruments and test how well they interrupt epileptic seizures in rodents. Prof. Schaffer will also assist with the design of the experiments and interpretation of results, as well as dissemination of findings to interested communities.

Substantial coordination of efforts will be accomplished via internet communications. The travel budget additionally includes support for two, face-to-face meetings of the key investigators during the duration of this phase of the project, one year.

Professor Dimitri M Kullmann, FMedSci FRS
UCL INSTITUTE OF NEUROLOGY
Department of Clinical and Experimental Epilepsy

10/08/2018

Re: SBIR grant proposal – Mouse-Sized Implantable Sensor with Lamp (MS-ISL)

This letter is to confirm my enthusiastic support for the proposal by Open Source Instruments, Inc, to develop and miniaturize a self-contained device to analyse electrocorticography signals and deliver light to the brain. Working with wireless transmitters developed by Open Source Instruments, my laboratory was the first to report that seizures in experimental animals could be suppressed optogenetically (Wykes et al., Sci Transl Med 2012 Nov 21;4(161):161ra1520). Since then we have made progress in automatic detection of seizures, new seizure models, improved targeting of neuron subtypes, and new gene therapy strategies that are amenable to clinical translation (Kätzel et al., Nat Commun. 2014 May 27;5:3847; Lieb et al., Nat Med. 2018 Jul 9. doi: 10.1038/s41591-018-0103-x). All of this work has relied heavily on devices purchased from Open Source Instruments. Our long-term goal is to use an entirely self-contained closed-loop device akin to automated implantable defibrillators used to detect and terminate cardiac arrhythmias. Similar technology could be applied to a variety of neurological disorders where abnormal brain rhythms are thought to play a role, including Parkinson's disease and schizophrenia. Our work to date has relied on rat models of epilepsy, with external computation of EEG patterns and fiber-optic light delivery. For us to make progress we would need a miniaturized device that could be implanted in a mouse, with built-in circuitry to control a light-emitting diode mounted on the skull. This would allow us to make use of the wide range of genetically modified mouse strains that allow optogenetic actuators (opsins) to be expressed selectively in different populations of neurons (either excitatory or inhibitory). There are also many mouse strains that have been validated as models of epilepsy, schizophrenia and other neurological disorders, further underlining the need to perform these experiments in mice.

I confirm that my laboratory would be keen to purchase the proposed Mouse-Sized Implantable Sensor with Lamp (MS-ISL) for implantation in mice. In the first instance, we would perform proof-of-principle studies that do not require the animals to be epileptic, to confirm that we are able to manipulate an observable behaviour (for instance turning when activating principal cells in the supplementary motor cortex). Furthermore, because of the inevitably limited battery life of implantable systems, we are keen for Open Source Instruments to enable the devices to be recharged.

The proposed development work represents important steps both towards clinical translation and towards testing circuit theories of brain function. I foresee many additional applications by our group and others.

In summary, I fully support the proposal by Open Source Instruments, and look forward to using the devices in my laboratory.

Yours sincerely,
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Yours sincerely

Dimitri M Kullmann
Professor of Neurology

National Hospital for Neurology & Neurosurgery • Box 101 • Queen Square • London • WC1N 3BG
PA: Jeni Byrd • ion.epilepsypa@ucl.ac.uk • ☎ +44 (0)20 3448 4194 • 🖷 +44 (0)20 7676 2155

Weill Cornell Medicine NewYork-Presbyterian

Theodore H. Schwartz, M.D., F.A.C.S.
David and Ursel Barnes Professor of Minimally Invasive Neurosurgery
Director, Anterior Skull Base and Pituitary Surgery
Director, Epilepsy Research Laboratory
Professor of Neurological Surgery, Otolaryngology and Neuroscience

Kevan Hashemi
President
Open Source Instruments
hashemi@opensourceinstruments.com

Dear Kevan,

I am writing to convey my willingness to serve as a consultant for your SBIR proposal with Chris Schaffer from Cornell University entitled, "An optogenetic brain implant with EEG monitoring and response for mice." As you know, I am a faculty member in the Neurological Surgery department at Weill Cornell Medical, where I see patients and run a research group focused on understanding and treating epilepsy. I have worked on several projects with Prof. Schaffer over the past ten years that have focused on using advance optical techniques to study seizure propagation and explore novel neurosurgical approaches to treating epilepsy in rodent models. For example, we have a paper that just appeared in Cerebral Cortex describing the efficacy of a laser-based surgical therapy to block the propagation of focally-initiated cortical seizures.

The device your company proposes to build in this proposal would be of great utility for the necessary animal studies that could guide future functional neurosurgical interventions in humans. Following neural activity patterns in one part of the brain, detecting aberrant activity patterns, and then delivering optogenetic stimulation (either excitatory or inhibitory) to modulate the activity patterns of a genetically-defined population of neurons in the same or another area of the brain is an emerging approach that has the potential to treat a wide variety of neurological and psychiatric diseases. To develop and test such therapies, we need the ability to do experiments like this in murine animal models. Your device exactly fills this need.

The decision to test the device in a rodent model of focal epilepsy makes sense to me, as it is clear in this model exactly where brain activity should be monitored and what cell types (and where) we should optogenetically modulate activity. My and Chris's lab have extensive experience with this model (in fact a former post-doc from my lab devised it) and I am happy to provide any technical assistance or advice needed to help you complete the proposed work.

I look froward to working with you on this exciting project.

Sincerely,

Theodore H. Schwartz

525 East 68th Street, Box 99, New York, NY 10065 | T. 212.746.5620 | F. 646.962.0118 | E.schwarh@med.cornell.edu | weillcornellbrainandspine.org

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Dr. Alfredo Gonzalez-Sulser
Centre for Discovery Brain Sciences
The University of Edinburgh
Hugh Robson Building
15 George Square
Edinburgh, EH8 9XD
United Kingdom
Telephone: +44-7706421230
Email: agonzal2@staffmail.ed.ac.uk

I am writing to establish my full and enthusiastic support for Open Source Instruments' application to the BRAIN Initiative to develop optogenetics for their wireless mouse electrophysiology systems. I am an independent researcher at the University of Edinburgh whose work is focused on testing optogenetic strategies to block seizures in rodent models of temporal lobe epilepsy (TLE). I am funded by Epilepsy Research UK, a charity in Great Britain dedicated to research in the disease.

I have personally used Open Source Instrument wireless electrodes and amplifiers to record from mice in various animal disease models over the past three years. The devices are very effective at recording from rodents continuously for 24 hours per day over weeks. This is wonderful for animal husbandry as long-term wireless recordings have less of an impact on the well-being of the animals than tethered protocols. Chronic wireless recordings are incredibly useful, as the field of epilepsy is shifting to models that better mimic the symptoms of the disease to improve our understanding of the mechanism leading to seizures and, to therefore have a greater chance of discovering novel translational approaches. One important characteristic of epilepsy is that seizures occur sporadically and are difficult to predict. Consequently, the use of chronic models of TLE, in which seizures occur spontaneously and often only once or twice per day, are becoming more prevalent in high impact publications. However, in order to detect these seizures and have sufficient samples for accurate statistics, it is necessary to record continuously over days or weeks.

Optogenetics allows us to modulate the activity of specific neuronal populations in experimental animals with a high degree of temporal precision. This is important as we can test how groups of cells may contribute to the epileptic circuit and determine whether these manipulations may have therapeutic value. These findings may eventually be translated to the clinic through deep brain stimulation of specific brain areas, genetic therapy or through implementation of optogenetics itself given significant future advances in these technologies.

Furthermore, genetic methods that allows us to access specific cell types and create novel models of disease with relative ease have been developed specifically for mice. In contrast, it is difficult and costly to generate rat and higher order mammalian models. Therefore, it is advantageous to utilize mice in epilepsy research and it would be very valuable to develop mouse wireless optogenetic stimulation, which is compatible with long-term chronic electrophysiology. This is a difficult proposition due to the small size of mice, limiting the size of devices, however given the developments of Open Source Instrument with rats it is highly likely they will succeed.

First generation wireless recordings and optogenetics systems have begun to be sold by multiple companies. However, very few of these technologies have the publication record that Open Source Instrument devices do. Furthermore, the majority of wireless technologies are designed for high sampling rate paradigms in order to record action potentials from individual cells. Consequently, the battery life of these systems is limited to a few hours. No clear solution has been designed yet for long-duration recordings in combination with optogenetics.

In conclusion, the field of epilepsy research, like other neuroscience sub-disciplines, is undergoing a revival thanks to the advent of optogenetics. The technology, in combination with genetic advances in mice has the potential to make important breakthroughs in deciphering the circuits underlying epilepsy and developing novel translational strategies for the disease. Furthermore, the specific technological advance proposed by Open Source Instruments, wireless optogenetic devices compatible with long-term electrophysiological recordings, is a critical element in order to advance this field of research.

Please do not hesitate to contact me if you have further questions.Yours sincerely,

Alfredo Gonzalez-Sulser, PhD
Epilepsy Research UK Fellow

UCL INSTITUTE OF NEUROLOGY
QUEEN SQUARE

THE NATIONAL HOSPITAL FOR NEUROLOGY AND
NEUROSURGERY
QUEEN SQUARE
LONDON WC1N 3BG

To whom it may concern,

I am a Senior Research Fellow at the UCL Institute of Neurology, in the Department of Clinical and Experimental Epilepsy.

I have been using telemetry devices manufactured by Open Source Instruments for the last 8 years and found them invaluable for my epilepsy research. These devices have allowed me to record EEG/ECoG continuously for months in freely moving rodents. I have found these devices reliable and robust.

Recently I have successfully implanted and tested ISL devices for *in vivo* optogenetic control of neurons in rats. I have successfully demonstrated that they can be used to drive population firing of neurons in the cortex to induce physiological behaviours (circling behaviour induced by 10Hz stimulation of opsin expressing neurons in the motor cortex, M2 area). I am currently using these devices in combination with an Open Source Instruments online seizure detection system to provide close-loop stimulation to suppress seizures in several rat models of neocortical epilepsy.

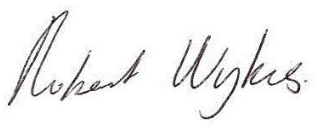
Future research aims will be to use miniaturised versions to allow similar experiments in mice.
There are 2 main reasons I would prefer to do some experiments in mice instead of rats and thus need the ISL miniaturised. Both are for transgenic reasons.

1. In rats we must use viral vectors to express an opsin and have limited ability to express our opsin in defined subsets or sub-populations of neurons. In mice we can use transgenic animals to express an opsin wherever we want it. There is much greater specificity and expression without the need to inject a virus.

2. I wish to conduct our experiments in animals that model human diseases. In our facility we have transgenic mice that model diseases such as Alzheimer's and Down syndrome both of which have abnormal EEG and hence the ability to manipulate neuronal firing *in vivo* using optogenetics is a research aim of ours. We also have other transgenic mice that harbour mutations in genes known in humans to cause epilepsy. These experiments can only be done in mice and not rats - hence the reason why we need a miniaturised ISL to meet our research aims.

I will use miniaturised versions of Open Source Instruments ISL devices when they become available to advance my telemetry research.

Yours sincerely,



Dr. Robert Wykes
UCL Institute of Neurology
R.Wykes@ucl.ac.uk
August 5th 2018

UNIVERSITY AT ALBANY
State University of New York

Annalisa Scimemi, PhD
Assistant Professor
Department of Biology

To: Brain Initiative, National Institute of Neurological Disorders and Stroke
Re: Open Source Instruments, Inc. SBIR Application

August 5, 2018

Dear Sir/Madam,

I am writing to express my enthusiastic support for Kevan Hashemi's application for a SBIR grant to support the commercialization of the implantable fiber-coupled LED for rodent optogenetics.

I am an Assistant Professor at SUNY Albany, with extensive experience in synaptic physiology, imaging and modeling. Recently, my lab has been in contact with Kevan Hashemi to purchase wireless transmitters to perform chronic EEG recordings from mouse pups. This is a challenging task not only because of the small size of the pups, but also because many of the recording tools available from other competitors are either wired or lack the ability to perform continuous recordings over the course of a week.

My lab is currently involved in a number of projects to determine how glutamate transporters shape synaptic, cell and circuit function in two regions of the brain, the hippocampus and the striatum, which play a fundamental role in the control of spatial navigation (the hippocampus), movement execution and reward (the striatum). Although performing EEG recordings allows monitoring the spontaneous electrical activity in the brain of living animals, we would like to expand our analysis to evoked responses triggered by activating implantable LEDs in the brain of genetically engineered mouse lines expressing the light-gated channel ChR2 under different cell-specific promoters. Through these experiments, we would be able to make direct inferences between the role of distinct classes of glutamate transporters and the execution of specific behaviors.

Therefore, I confirm that my lab would use the proposed mouse-sized Implantable Stimulator and Monitors with fiber-coupled LEDs. The development of the new technologies described by Kevan Hashemi in this proposal is, in my opinion, a fundamental step towards making discoveries that contribute to ameliorate the burden of mental health disease throughout the world. I fully support the proposal that Kevan Hashemi is submitting to your attention and I look forward to testing his new equipment in my lab. Should you require further information, please do not hesitate to contact me.

Sincerely,

Annalisa Scimemi

• **ADDRESS:** Biology Building, Room 329
1400 Washington Avenue, Albany, NY 12222-0100
• **PH:** 518-442-4367 • **FAX:** 518-442-4767 • **EMAIL:** scimemia@gmail.com or ascimemi@albany.edu
• **URL:** http://www.albany.edu/biology/people/faculty/fulltime/scimemi_annalisa.shtml
• **URL:** https://sites.google.com/site/scimemilab2013/