1. Significance

1.1 The Promise of Optogenetics

Epilepsy affects more than 50 million people [2,17]. Between 10-20% of these patients cannot be cured by current drug or surgical treatments [18,19]. Optogenetics is a new neuroscience research tool that has been helping researchers study the disorder and develop cures.

Optogenetics allows scientists to excite or inhibit selected subsets of neurons within the brain by applying flashes of light. The number of optogenetics papers published per year has grown from a dozen in 2009 to over five hundred in 2014 [10]. This advance has buoyed epilepsy research [18,20].

Epilepsy is characterized by hyperactive neural circuits. Optical stimulation of targeted neurons has been shown to reduce the severity of epileptic activity and the frequency of events [2,21]. **OSI's collaborators and other groups have used light stimulus in a closed-loop system to abort seizures in rodent models, akin to how implantable defibrillators treat cardiac arrhythmias** [2,3,22,23,24].

Similar closed-loop systems may also be used to treat a variety of neurological disorders where abnormal brain rhythms are thought to play a role, including Parkinson's disease and schizophrenia. Optogenetics is already being used to study these diseases [25,26,27,28].

1.2 The Role of Instrumentation

In vivo optogenetics experiments have conventionally relied on tethered instruments. These devices use an optical fiber to deliver light from an external laser or LED to the rodent model's brain. Similarly, equipment used to record neural activity relies on a wire to deliver the data to an external device. While this equipment has been suitable to demonstrate the power of optogenetics, it is severely limited for several reasons.

Any physical connection between a laboratory animal and external equipment is a mechanical constraint upon the movement of the animal and creates major challenges for the cohabitation of animals. Many national and institutional animal welfare guidelines limit the use of tethers to a few hours per day, or to times when the animal is being observed by a nearby human being. The physical tether is incompatible with enclosed testing environments such as Pre-pulse Inhibition (PPI) chambers or operant boxes, making many experiments completely impossible.

Several groups have attempted to address these issues by developing wireless systems [Table 1]. However, **none of these wireless systems are able to record EEG signals or otherwise measure biopotential**. This makes many desirable experiments impossible to conduct, including all of the closed-loop therapy techniques described above. Current wireless systems are also subject to major practical shortcomings including **short standby time, necessary supervision, and commercial non-availability**.

We propose to build a wireless, subcutaneous optogenetic instrument which will solve the technical shortcomings that currently frustrate neuroscientists. In addition to making conventional experiments much more practical, our device will enable a new range of experiments for both epilepsy and other diseases that require ***i)*** **free animal behavior;** ***ii)*** **real time recording of EEG signals; and** ***iii)*** **the use of enclosed spaces (operant boxes, T mazes, etc)**. We will make the instrument reusable and readily available at an affordable price of approximately $600 per unit.

2. Innovation

2.1 Biometric Instruments at Open Source Instruments Inc. (OSI)

Open Source Instruments Inc. (OSI) was founded in 2004 to design equipment for scientific research [9]. OSI distributes its circuit diagrams, data acquisition software, and documentation for free under the GNU Public License [7].

Soon after its founding, OSI entered a collaboration with Matthew Walker, Institute of Neurology (ION), University College London (UCL), to design and develop an implantable, wireless EEG

monitor for epilepsy studies in rats. One of the primary motivations for ION to enter into this collaboration was the fact that the telemetry system and data acquisition software would be open source, so that ION could modify the system to suit their own needs in the future. After five years of collaboration, OSI demonstrated an effective and reliable wireless EEG monitor [6,15]. Our monitor produces recordings free of noise and artifact, which makes automatic event detection possible in long-term recordings. These devices have enabled several successful and original studies in epilepsy [2,3, 4,5]. We have sold over 2000 subcutaneous, wireless EEG monitors for both rats and mice since 2010.

In 2013, OSI began developing an optogenetic stimulus capability for its rat-sized EEG monitor. We developed this device in collaboration with Dimitri Kullmann (ION, UCL) and call it the Implantable Sensor with Lamp (ISL) [16] [Figure 1]. **The ISL 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 stimulus.** The device does not use an external tether during any stage of deployment.

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.

The ISL has been implanted in rats and is proven to provoke an optogenetic response (behavioral changes and induction of seizure) [8]. Preliminary results indicate success as a closed-loop device for aborting automatically detected seizures [30].

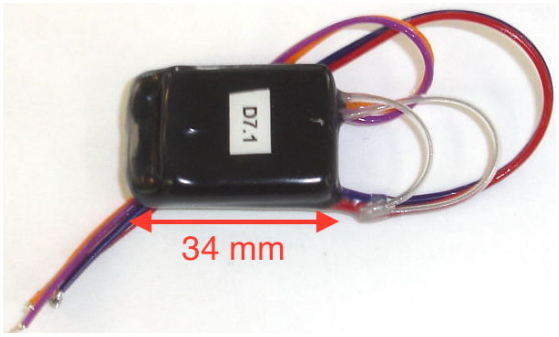

Figure 1a: OSI's current Implantable Sensor with Lamp (ISL) is used to record EEG signals and drive optogenetic stimulus in rats. This component is implanted in the abdomen where it doesn't interfere with animal behavior. Electronics and battery are enclosed in the black epoxy package. The silver loops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

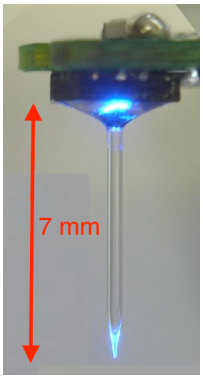

Figure 1b: The Fiber Coupled LED (FCL) is the satellite head fixture driven by the ISL. It is mounted to a hole in the skull with dental cement. Blue light is visible at the LED surface and at the coupled fiber's tapered tip. The sharp point minimizes neural scar tissue and maximizes the volume of tissue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

2.2 The Need for a Mouse-sized Device

Following the success of our rat-sized ISL, researchers have requested a similar device that can be used with mice. When rats are used in optogenetic experiments, a viral vector must be used to express the opsins that make neurons sensitive to flashes of light. This method is slow, imprecise, and requires expertise not available to many laboratories. By contrast, a wide variety of transgenic mouse strains are 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**. Our letters of support emphasize this point.

2.3 Survey of Current Optogenetic Technology and OSI's Difference

Several companies provide optogenetics technology which at first appears suitable for the transgenic mouse experiments described above. Several vendors provide tethered products. The tether inhibits free animal movement, requires frequent operator handling and supervision, limits the amount of available experiment time per day to a few hours, is incompatible with enclosed testing spaces such as operant boxes, and is susceptible to recording artifacts produced by cable movement. To overcome the shortfalls of tethered equipment, several groups have developed

wireless instruments that can deliver optogenetic stimulation to mice [12,29,31,32]. They are compared to our proposed instrument in Table 1.

None of the competing devices support EEG signal recording nor any other biopotential measurements. We will incorporate our EEG measurement technology into the proposed instrument. Its performance will be very similar to that of our rat-sized ISL [Figure 2]. **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. 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. OSI's telemetry system provides robust reception.

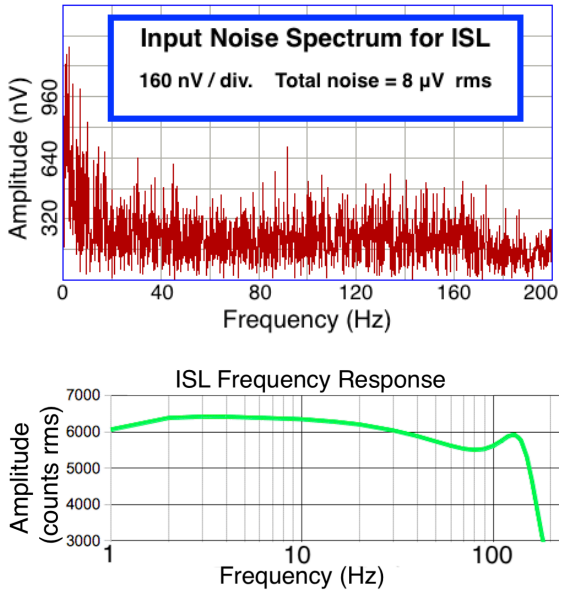

Figure 2 - Biopotential performance
The proposed instrument will have EEG recording characteristics very similar to our current rat-sized optogenetic instrument. **Top:** Input noise spectrum for the rat-sized ISL **Bottom:** Frequency response of the rat-sized ISL. A three pole filter is used to limit the contribution of signals above 160 Hz.

Most competing wireless optogenetic equipment relies on wireless power transfer to run the instrument [12,13,29] because their electronics are not efficient enough to be powered by a battery. The contact between brain tissue and an EEG electrode demodulates the wireless power oscillations and produces low-frequency artifacts in the EEG recording. We have observed this effect in our laboratory with radio frequency signals that are one million times less powerful than those that are required for a power system. **Wireless power systems are certain to irrevocably corrupt EEG signals in all cases.** Wireless power systems also suffer low reliability due to mouse movement disrupting power flow.

We at OSI believe that the only way to make a practical implantable optogenetic stimulator with monitoring capability is to provide power with a battery. OSI has proven this approach to be effective with the rat-sized ISL. 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 125 days of standby time and 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 instead of 24, the device will run for 45 days.

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 [29]. 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.**

Table 1: Survey of Wireless Optogenetic Devices						
	OSI's Proposed ISM & FCL	**OSI's current A3030D (ISL)**	**Kendall Research (Firefly) [12]**	**Teleopto (TeleR-1-P) [32]**	**Riken Brain Science Institute [31]**	**Ada Poon's Group, Stanford [29]**
EEG monitoring	Yes	Yes	No	No	No	No
Location	subcutaneous	subcutaneous	head mounted	head mounted	head mounted	subcutaneous
Volume	1.5mL	4.2mL	~3mL	1.6mL	~2mL	0.1mL
Power Source	Rechargeable Battery	Battery	Electro-magnetic	Rechargeable Battery	Rechargeable Battery	Electro-magnetic
Standby Time	3000 hours	10000 hours	NA	17 hours	20 hours	NA
Programmable Stimulus	Yes	Yes	Yes	No	No	Not while cohabiting
Independent addressing	Yes	Yes	Yes	Yes	Yes	No
For Sale	Planned	Yes	Yes	Yes	No	No

Another advantage of OSI's system is our classification and analysis software. OSI's Neuroarchiver Tool has proven so successful that even groups who do not use OSI telemetry are using it to count ictal events in their recordings. The Neuroarchiver will record and analyze EEG data from the proposed device. The Neuroarchiver includes an automated event classifier which has been used to automatically detect epileptic events in both live data and during the playback of thousands of hours of recorded data [2,3]. We continuously publish improved data analysis algorithms to satisfy new customer requirements. The Neuroarchiver is well-documented, free, and open source.

The optical stimulus of the proposed instrument will be controlled by software developed for the rat-sized ISL. The default mode of optical stimulation is a train of pulses. Researchers may select the light intensity, number of pulses, pulse length, and pulse spacing. There is also support for randomization of the pulse train. We will lightly modify the existing optical control software to make it compatible with our proposed instrument. **In an ongoing *in vivo* study by one of our customers, the Neuroarchiver Tool automatically detects seizures and then triggers the optical control software to apply stimulation [30].** The Neuroarchiver and optical controller both support custom scripts which give researchers full control over their experiment.

Overall, 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. The fully implantable design means that animals are free to cohabit in natural social structures without the fears of mutilation that limit other devices. The combination of battery power and proven telemetry system make the system completely reliable.

3. Approach

Aim 1: Develop the electronics for a mouse-sized optogenetic stimulator with monitor

Milestone 1: Reduce the volume of OSI's current ISL electronics from 4.2 mL to 1.5 mL

We will use the current ISL circuit board as a starting point for the ISM 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 2.6 mm on each side. This substitution alone will reduce the volume of the device by 0.95 mL when accounting for the amount of circuit board and bioconformal coating that will be saved. Table 3 estimates the amount of circuit board surface area which will be saved by similar substitutions. The largest volume savings will be achieved by replacing the current 2.4 mL lithium ion cell with a 0.3 mL lithium polymer cell. Estimated runtime with this battery is shown in Table 2.

We will further reduce the volume by eliminating one of the two antennas in our present design. Currently, separate antennas are used for command reception and data transmission (50 mm and 30

mm long respectively). We will incorporate a standard antenna switch into the new circuit which will allow a single 30 mm antenna to fulfill both functions.

We will design a new circuit board layout to accommodate the smaller components. The total volume of the board and battery will be 1.5 mL or less once assembled and coated.

Milestone 2: Add a recharging capability which permits the instrument to be reused

Our current optogenetic instrument is designed to be used for a single experiment and then disposed. Priced at $500, this is an accessible option for many labs. Still, we plan to offer higher value in the proposed device by making it re-usable. Currently, researchers choose to re-use some of our similarly built EEG transmitters, so the process is well understood.

After the mouse is sacrificed, the instrument can be recovered surgically and/or by dissolving the animal in acid. The dental cement which attaches the head fixture to the skull may be dissolved with acetone. The leads which connect the main circuit board to the LED may then be plugged into a charger.

We will develop a charger that monitors the state of the battery and replenishes it to full charge. Within the ISM, we will include an arrangement of diodes that permit its battery to be recharged using the LED leads. Once charged, the instrument will be ready for use in a new experiment.

Table 2: Predicted runtime for the ISM

Operation Mode	Average current draw	Runtime per charge
Standby Mode	5 µA	> 125 days
Optical stimulation for 30 minutes per day	28 µA	30 days
Continuous EEG recording	75 µA	10.5 days
Epilepsy Experiment	84 µA	9.5 days

Runtime for the proposed device is estimated based on current consumption measurements taken for our existing rat-sized ISL device.
Standby mode: the device is inactive but able to receive commands and start activity
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 [30].
Continuous EEG recording: EEG signals are being both recorded and transmitted to the base station in real time at 512 samples per second for 24 hours per day.
Epilepsy Experiment: Uses 24 hours of EEG recording per day and closed loop optical stimulation in response to 12 seizures per day (total of 12 minutes of optical stimulation per day).

Aim 2: Develop an ultra-efficient, fiber-coupled LED (FCL) appropriate for mice

Milestone 3: Develop an FCL circuit board using the DA2432 LED die

One of the largest barriers to creating a mouse-sized optogenetic instrument is the challenge of delivering sufficient optical power to activate photosensitive 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 solved this problem in the rat-sized instrument 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)* tapering the optical fiber to maximize radiant flux and minimize the formation of opaque scar tissue. In a mouse-sized instrument, the constraint on available energy is even more severe due to the smaller battery size, and the challenge is further exacerbated by the need to reduce the diameter of the fiber itself for use with mice. We must achieve further improvements in efficiency to make the mouse-sized device practical.

Our current LED (Cree EZ-500) is larger than the cross section of the coupled fiber, so some light is lost from the edges of its surface. For the proposed instrument, we will switch to a smaller LED which will minimize this source of loss. The new LED must be available as a bare die for efficient fiber coupling as opposed to being sold pre-packaged in clear epoxy as is the usual case. The Cree DA2432 satisfies our requirements. Independent of geometric gains from superior geometry, it also offers more optical power than the EZ500 for the same amount of electric power.

The DA2432 is significantly more challenging to work with than the EZ-500 since its package is just 240 x 320 µm across and it cannot be wire-bonded into the QFN-8 holder package that we

currently use. Instead, it must be soldered to a custom circuit board with specialized equipment. We have consulted with a reliable supplier who is confident in their ability to mount DA2432s for us using their equipment. We will design a new circuit board for the FCL and have a batch of boards manufactured with the DA2432 mounted by machine. In addition to improving the instruments' optical capture efficiency, changing LEDs will allow us to reduce the head fixture size by at least 2 mm in each dimension.

Table 3: Space savings achieved by part substitutions

Component	Purpose	Area (mm^2)	Substitute area (mm^2)
LCMX02 – 1200	Logic Chip	256.0	6.9
NTR4003N	Transistor	7.8	1.0
SN74AUP1G08	Logic Gate	4.0	0.6
SN74AUP1G32	Logic Gate	4.0	0.6
TPS70930	Voltage Regulator	4.7	4.0
TPS70912	Voltage Regulator	4.7	4.0
20 capacitors		10.0	3.6
22 resistors		11.0	3.9
	Total:	**302**	**25**

Many of the components used in the rat-sized ISL can be replaced with similar or identical parts that are available in smaller packages. The table lists the components which we will substitute, their area, and the area of their replacement.

The head fixture used by our current ISL includes an LED, optical fiber taper, and guide cannula for drug delivery. Since transgenic mice do not require lentivirus injections into the brain to express opsins, the guide cannula will no longer be necessary as a standard option for the FCL, further saving volume and weight. The new board will account for this change.

Milestone 4: Develop 220 µm diameter fiber tapers and measure optical power

The rat-sized ISL uses optical fiber tapers with a base diameter of 450 µm. This is too large for mice, so we will develop new algorithms for our taper-pulling machine to craft 220 µm diameter fiber tapers. We will modify the taper-pulling machine to be able to grip the smaller fiber, and we will machine custom holders for polishing the 220 µm diameter 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.

We will mount the 220 µm diameter tapers to the FCL board and measure optical power output. The 220 µm diameter fiber has only 22% the light gathering surface area as the 450 µm diameter fiber which we currently use. This large loss will be compensated by the improved LED efficiency and geometry described in Milestone 1. The current FCL provides 13 mW of light at its default operating current of 40 mA. The proposed instrument will produce at least 4 mW of 460 nm light from its fiber tip at the same current. Even 2 mW is sufficient to activate channelrhodopsin-2 and halorhodopsin molecules in mammalian neurons [33]. The LED can be driven at a different intensity or duty cycle as necessary.

3.3 Aim 3: Manufacture prototype instruments and conduct an *in vivo* trial

Milestone 5: Manufacture 20 instruments We will assemble 20 fully complete instruments including the application of our bioconformal coating made of epoxy and silicone.

Milestone 6: Conduct local tests

We will perform local tests on 10 of the instruments to ensure quality and reliability. Tests include placing them in accelerated aging environments with high heat, 100% humidity, high salinity, and random motion. We will publish full characterization of performance and reliability.

Milestone 7: Conduct an in vivo trial to assess performance

We will ship the other 10 prototypes to our long-time collaborators at the Institute of Neurology (ION), University College London. They will implant the devices in mice and report on: *i)* the device's ability to manipulate observable behavior in response to optogenetic stimulus; *ii)* the device's ability to record EEG signals; and *iii)* device reliability. We expect all ten devices to remain functional at the end of the 12 week trial, thereby indicating sufficient reliability for commercial viability. Our collaborators will recover at least two of the instruments from sacrificed animals after the trial period to demonstrate that the instruments can be recharged and reused.