








Development of Optrodes and Instrumentation for Wireless Optogenetic Application

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Abstract: Optogenetics combines optical and genetic techniques to control and monitor neuronal activities. Recent efforts focus on developing portable and wireless electronics for optical activation and biopotential acquisition. These advancements aim to offer greater mobility and freedom for studying animals, contrasting with the large equipment commonly found in laboratories for laser activation, signal amplification, and data acquisition. This study presents the development of a wireless optrode system for optogenetics, integrating optical stimulation and biopotential acquisition in a compact, portable format.


1 INTRODUCTION


Genetic engineering techniques are used to allow neuronal populations to produce light-sensitive proteins. These proteins can be stimulated or inhibited by specific light patterns and wavelengths. Deisseroth et al. (2006) defined optogenetics as a technology that “combines genetic targeting of specific neurons or proteins with optical technology for imaging or control of the targets within intact, living neural circuits”. To validate neuronal responses to light stimulation, electrodes and techniques such as patch-clamp electrophysiology can be employed, allowing precise measurements of intracellular and synaptic activity evoked by optical stimuli, as demonstrated by Boyden et al. (2005).


In the current context, optogenetics applications are generally carried out in laboratories equipped with large optical equipment and instruments for reading signals. Compact systems are being developed to address limitations such as the need for wiring and dependence on multiple pieces of equipment (light emission controllers, acquisition hardware and independent implantable parts), promoting greater mobility in animal studies and allowing their free behavior. The next chapters will address the development of an optical stimulation system that integrates with a biosignal acquisition system and features an optrode module for use with an embedded biosignal acquisition and optical stimulus control system.


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2 OPSINS

Opsins are essential tools in optogenetics, allowing neuronal circuits to be stimulated or inhibited by light. “Opsins are membrane-bound proteins that are activated by light, which results in activation (depolarization), inhibition (hyperpolarization), or modulation of the intracellular signaling cascade” (Guru *et al.*, 2015). Opsins function as ion channels or pumps that are activated by specific wavelengths of light. “Upon activation by light, these channels and pumps respond by opening or closing, which conducts the flow of ions into or out of the cell” (Addgene, n.d.). Three commonly used opsins include:

(1) Channelrhodopsins: channel-type opsins that allow rapid depolarization of neurons through direct stimulation of ion channels when exposed to light (Addgene, n.d.). Channelrhodopsin-2 (ChR2) is activated by light with wavelengths in the range of 400nm to 500nm (Dufour *et al.*, 2015).

(2) Halorhodopsins (NpHR): opsins that pump chloride ions into the neuronal membrane, causing cellular hyperpolarization and inhibiting neuron activation. NpHR is activated by light with wavelengths in the range of 550nm to 620nm (Dufour *et al.*, 2015).

(3) Archaeorhodopsins: light-controlled opsins that pump protons out of the neuronal membrane, causing cellular hyperpolarization and inhibiting neuron activation. ArchT is activated by light with wavelengths in the range of 500nm to 600nm (Dufour *et al.*, 2015).

In this way, an optrode can be equipped with different light sources to suit the use in conjunction with the different opsins used for different studies and purposes.

3 SYSTEM ARCHITECTURE

The optrode developed in this project was designed for use in a wireless system for optogenetic stimulation and signal recording. The system is compact, battery-operated, and integrates a microcontroller with wireless communication, along with circuits such as an analog-to-digital converter, auxiliary sensors, and a power management system. The acquisition system connects to the optrode via a board-to-board connector.

The structure of the acquisition system is represented in Figure 1.

3.1 Optrode Module

The optrode module connects to the acquisition board, receiving the necessary power, the communication buses and delivering the analog signals to the ADC (analog to digital converter) in the other board. Its architecture is shown in Figure 2.

The board incorporates a signal filtering stage to ensure adequate visualization of the read signals, a temperature sensor and auxiliaries to monitor the activity of the study animal, in addition to means of controlling the light pattern. As optrode module design requirements, the following items have been listed:

- (1) It must have a means of delivering light;
- (2) It must have an interface with electrodes;
- (3) It must provide rapid integration and configuration with the acquisition/control system;
- (4) It must provide means for optical control of excitation patterns;
- (5) It must provide the necessary energy for the components;
- (6) It must provide an adequate analog interface;
- (7) It may include additional sensors.

3.1.1 Implantable Interface

To stimulate and measure the activity of a test animal, it is necessary to perform optical delivery and reading of the biopotentials of interest. Signal acquisition is generally achieved through electrodes. As the optrode module provides signal delivery to the acquisition board's ADC, it is possible to adapt the module to capture signals from other sensors, such as pressure and temperature sensors, just by modifying the interface with the test animal and without interfering with the main acquisition and control module.

Light delivery can be done by optical fiber, or, as adopted in this project, by small SMD LEDs.

4 DEVELOPMENT

4.1 Light Emitter

To stimulate or inhibit neurons expressing opsins, light at the appropriate frequency and intensity must reach the target. Each type of opsin responds to a specific wavelength of light, as mentioned previously. For ChR2 photostimulation, Chen *et al.* (2016) mentions pulse widths between 1ms and 15ms as typical requirements, pulse

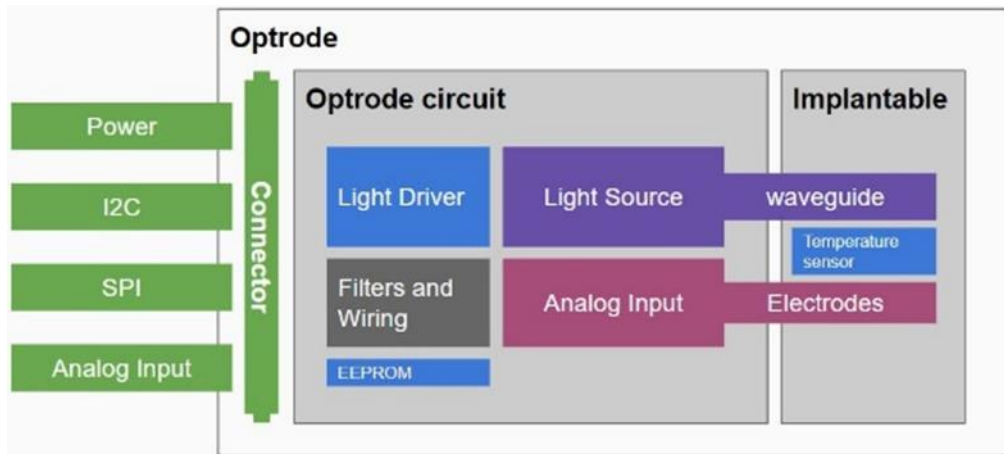


Figure 1: Optrode module architecture.

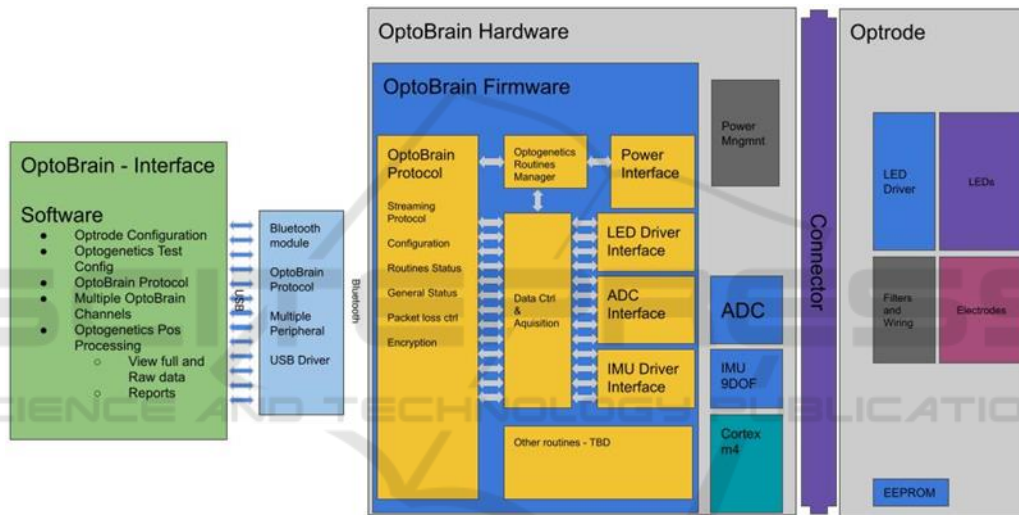


Figure 2: System architecture for optogenetics.

frequency between 1Hz and 50Hz, and an optical power at the fiber tip of at least $32\mu\text{W}$. A minimum irradiance of $1\text{mW}/\text{mm}^2$ when delivering light to neurons via an optrode (Freitas *et al.*, 2021).

There are three common types of light sources used for excitation in optogenetics: arc lamps, lasers, and LEDs. The work by Lin (2012) mentions the following characteristics for each of the light sources:

- (1) Arc lamps: provides a continuous spectrum of wavelengths in the visible range. However, lighting control is done via a shutter, limiting the stimulation frequency.
- (2) Lasers: provides high-intensity light that can be coupled to an optical fiber. It usually involves a high equipment cost and wavelengths are limited
- (3) LEDs (light-emitting diodes): physically smaller than other options and can be mounted

directly on the brain. Due to its size and current, it can generate heat. Its intensity is typically weaker compared to the other options.

In this project, the use of LED was chosen to meet the need to obtain a compact and low energy consumption system in a wearable device. To interface the optrode module in delivering light to the test animal, an arrow-shaped printed circuit board with a width of 0.7mm and a pointed end was manufactured. Figure 3 shows a photograph of a prototype of the implantable interface, which has only two tracks and pads for soldering the LED, near the pointed end, and for soldering wires to connect to the optrode module, at the other end. As it has two terminals, it is also possible to use the same board design as an electrode to read potentials. The total length is 20.7mm and 0.5mm thickness.

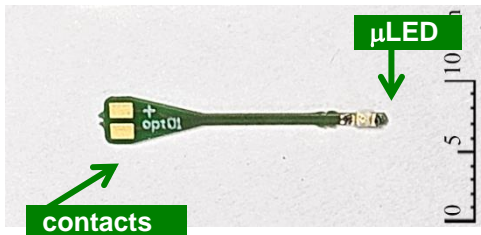


Figure 3: Photograph of an optrode module prototype with Dialight 598-8091-107F LED.

4.2 LED Driver Circuit and Other Components

The TLC5940 integrated circuit from Texas Instruments was chosen to be used to control the LEDs. Key characteristics that influenced this choice include:

- (1) Capability to control up to 16 LED channels;
- (2) Programmable and individual current control for each channel in 64 levels;
- (3) 4096-level individual PWM control;
- (4) Compact size, 5.00mm×5.00mm in VQFN version;
- (5) Serial communication up to 30MHz.

The integrated circuit's communication protocol does not follow a recognized standard, however it is possible to use SPI with some modifications. Additionally, a WLCSP-4 package EEPROM measuring 0.77mm×0.77mm was included in the design for quick recognition and configuration by the acquisition board. To validate the project, a 20 mm x 20 mm printed circuit board was manufactured to interconnect the aforementioned components. The following were also included:

- (1) Solder pads for 2 LEDs;
- (2) Solder pads for 2 signal reading pairs, which can be connected to an electrode, temperature sensor, or both;
- (3) Solder pads for resistors and capacitors on the analog signal tracks for RC filter implementation;
- (4) 40-pin connector, on the back of the board, for connection to the acquisition module;
- (5) Electrical test points to facilitate testing.

The optrode module, together with the interface board is shown in Figure 4, while Figure 5 shows the optrode module coupled to the acquisition board.

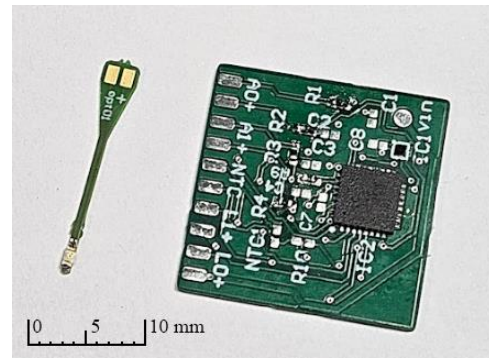


Figure 4: Optrode module and interface board.

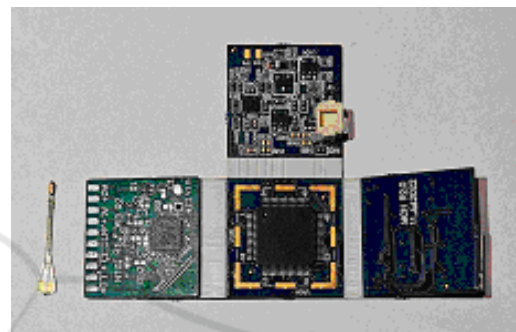


Figure 5: Optrode module connected to the main acquisition module.

5 BENCH TESTS

The optical power of commercial LED models and the performance of optical pattern execution for optogenetics applications were evaluated under bench test conditions

5.1 Optical Power Test of LEDs

A search for LED models was conducted, and five models were selected and tested. For the selection, only models with an emitted wavelength close to 473 nm—the region in which CHR2 is maximally activated—were considered.

The current of each LED was adjusted to close to its nominal value and close to its maximum value by varying a potentiometer to adjust the current while it was measured by a Fluke 287 multimeter, and the optical power was recorded using the Thorlabs PMD100D Optical Power Meter settled to 473nm wavelength in an environment with a dark chamber.

5.2 Optical Pattern Execution Tests for Optogenetics

Figure 6 illustrates a structure that was created to describe the pulses to be executed. The parameters that can be configured when defining stimulation pulses are listed below:

- (1) Amplitude: The amplitude of the optical pulse will be given by the current control, being directly related to the light intensity
- (2) Delay: Delay in starting activation;
- (3) Width: Length of activation time;
- (4) Interval: Time interval between activations;
- (5) Pulse Number: Number of train pulses;
- (6) Repeat Interval: Time interval between repetitions;
- (7) Repeat Number: Number of repetitions.

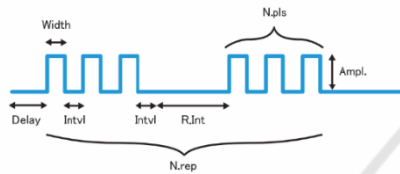


Figure 6: Description of a pulse pattern.

The optrode module received commands to reproduce the following pulse pattern:

- (1) Delay: 500ms,
- (2) Width: 2ms,
- (3) Interval: 500 μ s,
- (4) Pulse Number: 5,
- (5) Repetition interval: 2s,
- (6) Number of repetition: 3.

To check the optical pulses, a circuit with a photodiode and a resistor in series was assembled, so that the reverse current generated in the photodiode when sensitized by the pulse pattern of the optrode module generates a variation in the resistor voltage. Figure 7 illustrates the schematic of this circuit. The voltage across the resistor is monitored on an oscilloscope, and the shape of the generated wave is compared to the pulse pattern performed.

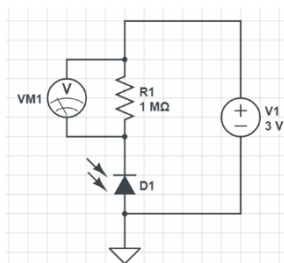


Figure 7: Schematic of the optical sensor circuit for testing pulse patterns.

6 EXPERIMENTAL RESULTS AND DISCUSSION

6.1 Optical Power of Commercial LEDs

In total, five LED models were tested. Each of them has been tested close to its rated current and its maximum current, when possible. Figure 8 shows the LED approach to the sensor in the image on the left and the measurement panel on the right. Table 1 summarizes the results obtained.

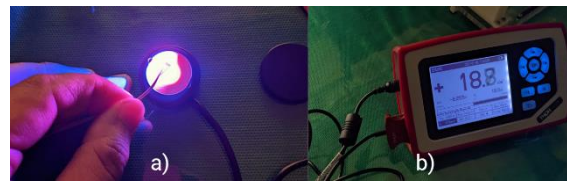


Figure 8: Optical power test: (a) sensitive component and (b) power measurement.

The LED model DA2432, manufactured by Cree, demonstrated high light power (18.7mW) despite its small physical size. Furthermore, its maximum current is 100mA. However, its compact dimensions (240 μ m by 320 μ m) make soldering challenging and increase the risk of losses. Additionally, obtaining more units of this model was not possible, as it is an obsolescent product. Cree's CLM3A-BKW-CUAVA453 model exhibited similar optical power levels at a lower current, but its volume is approximately 652 times larger. Roithner's B5B-437-IX model delivered slightly more than half the optical power of the previous models and has the largest physical size among them. Dialight's 597-3601-207F model presented the lowest optical power even though it was larger than the 598-8091-107F model from the same brand, which, in turn, obtained 5.15mW of optical power and the second smallest size among them.

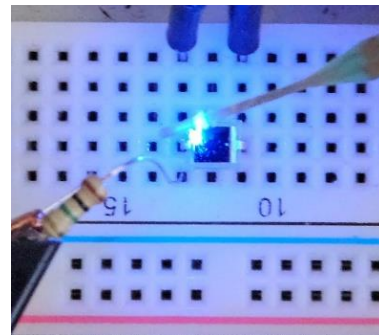


Figure 9: Measurement of the performed pulse pattern.

Table 1: Measurements of LED power.

Supplier/Model	Dimension [mm]	Wavelength [nm]	Nominal current [mA]	Measured optical power [mW]
Cree DA2432	0.24×0.32×0.14	470	20	5.6 @ 19.6mA 18.7 @ 99mA
Cree CLM3A-BKW-CUAVA453	2.7×2.0×1.3	470	20	18.8 @ 19.6mA 10.4 @ 10.0mA
Dialight 598-8091-107F	1.6×0.8×0.7	473	20	5.15 @ 19.6mA
Dialight 597-3601-207F	3.5×2.8×1.9	465	20	1.7 @ 20mA
Roithner B5B-437-IX	5 (diameter)	468	30	8.3 @ 21mA 10.6 @ 30mA

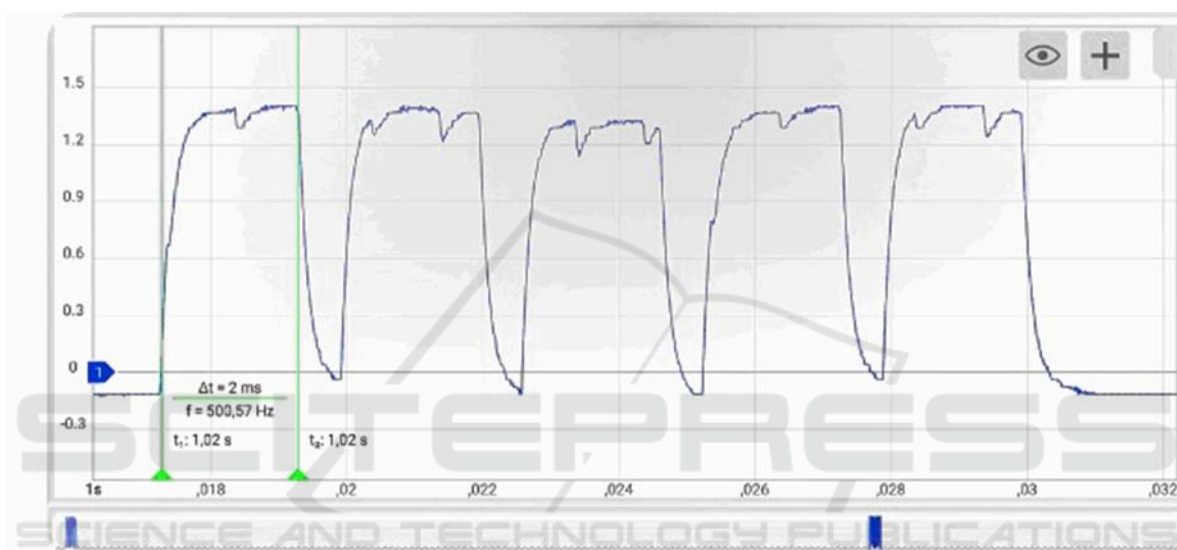


Figure 10: Train of pulses captured by the oscilloscope.

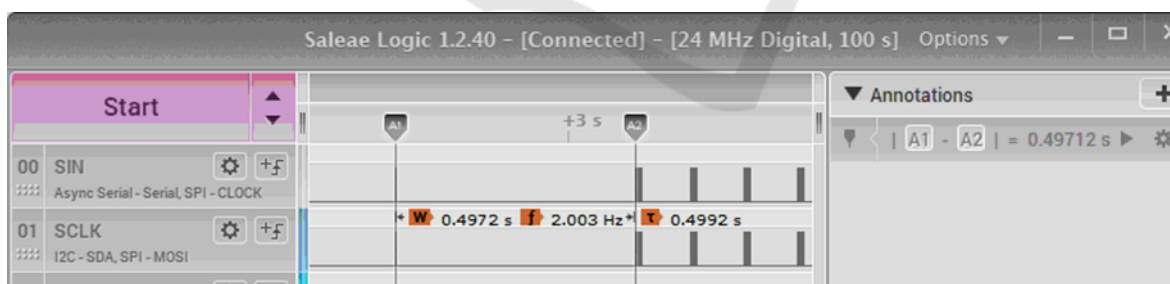


Figure 11: Programmed delay time measured in logic analyser.

6.2 Execution of Optical Patterns for Optogenetics

The photodiode was assembled in series with a resistor, as shown in Figure 7. Figure 9 shows the assembly of the photodiode and the approach of the interface board to the LED performing the pulse patterns. The LED used in the tests was the Dialight 598-8091-107F.

The voltage across the resistor was measured using a Hantek 6022BL digital oscilloscope. The captured pulse train is presented in Figure 10, where the pulse width, inter-pulse interval, and repetition times were analyzed.

The start delay (delay parameter) was defined as the time interval between the last clock pulse of the TLC5940 initial configuration transmission and the beginning of the first pulse transmission. This

measurement was performed using a Hantek 6022BL logic analyzer in conjunction with Saleae Logic 1.2.40 software. The results of the configured and measured parameters are summarized in Table 2.

Table 2: Configured values and measured values when executing pulse patterns.

Parameter	Configured	Measured
Delay [μ s]	500	497
Width [ms]	2	2
Interval [μ s]	500	595
Pulse Number	5	5
Repetition Interval [s]	2	1.98
Repeat Number	3	3

7 CONCLUSIONS

In bench tests, the optrode module successfully reproduced the patterns required for optogenetics experiments, demonstrating both adequate optical power and temporal precision. These results support the continuation of the project's development. The next steps include full integration and testing with the acquisition electronics, enabling simultaneous stimulation and signal acquisition. Subsequently, in vivo tests will be necessary to validate the system's performance in practical scenarios. Additionally, developing alternative interfaces for light delivery and signal capture, as well as exploring other wavelengths, is of interest to expand the system's applicability to diverse contexts.

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