[2020 Winter/Spring semester URP] KAIST URP Program

Individual Research Project Final Report

Research Participation Project

실시간 신경 회로 컨트롤 및 모니터링을 위한 무선 유연성 뉴럴 프로브 개발

Wireless flexible neural probe for real-time control and monitoring of neural circuits in freely moving animals

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For Submission

Research Subject (Korean) : 실시간 신경 회로 컨트롤 및 모니터링을 위한 무선 유연성 뉴럴 프로브 개발

Research Subject (English) : Wireless flexible neural probe for real-time control and monitoring of neural circuits in freely moving animals

Research Period : January 2, 2020 ~ July 3, 2020

Advisory Professor : Jae-Woong Jeong (Signature)

Teaching Assistant : Juhyun Lee (Signature)

As a participant of the KAIST URP program, I have completed the above research and hereby submit the final report on the research.

July, 8th, 2020

Researcher Bella Godiva (Signature)

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Abstract

Existing neural circuit stimulation and neural recording tools mostly are still made of rigid materials which might cause damage to the surrounding tissues. The majority of the available technology is still being operated using wires and this significantly limits animals' free behavior and movement as well as limiting the scope of brain research experiments. Optogenetics is an emerging neuroscience tool to manipulate neurological system with high spatiotemporal precision. However, neural stimulation by means of optogenetics and neural recording is usually achieved using separate modules of optical fiber, a metal cannula, and a recording probe. The combination of all these large size objects might cause large tissue damage and using a separate module might result in spatial mismatch between points of light emission and neural recording. Here, we present a wireless flexible neural probe that succeeds in achieving real-time simultaneous control and monitoring of neural circuits in freely moving animals. 3D-printing based fabrication method is utilized to produce low-cost optogenetic neural probe and MEMS process is applied to fabricate transparent probe for neural recording and photostimulation and recording of neural circuits. A PC-based user interface will be developed to control microscale inorganic light-emitting diodes (μ-ILEDs) on the neural probe while displaying brain signal data. Bluetooth low energy (BLE) is chosen to support wireless communication between neural implant and PC. Several experiments are conducted to test this configuration and the results show that real-time wireless control and monitoring are achievable. Further enhancement of the user interface may be done to provide analysis of received brain signals.

1. Research Background

Unveiling the mystery of the brain can help in improving our understanding of brain disorders and neurodegenerative diseases, opening new opportunities for the discoveries of more effective therapeutic methods and remedies for those diseases. Therefore, there is a demand today for generating and enhancing neural recording and stimulation tools which could allow individuals to study neural circuit dynamics. Conventionally, this neural circuit stimulation and neural recording tools are made of rigid materials such as silica and metals. Although effective for short-term, mechanical mismatch between the soft brain tissue (elasticity modulus (E) ~ 0.1–6 kPa) and the stiff devices (E > 150 GPa) can cause glial scars or neuron-free death zone surrounding the implant, making it unsuitable for chronic use. Advances in microfabrication shades light in overcoming this issue as the usage of various materials (e.g., silicon, SU-8, polyimide (PI), etc.) to build thin, flexible and highly biocompatible implants starts emerging.

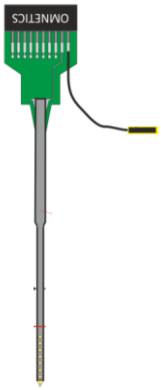


Figure 1. Rigid metal-based neural probes

Figure 2. Thin and flexible neural probes

Optogenetics - the use of light to manipulate neural activity, is an emerging neuroscience tool to selectively examine and control neural circuits and systems in conscious, freely moving animals. This ability to control neuronal activity with unprecedentedly high spatiotemporal resolution has led to breakthroughs in our understanding of mammalian brain function and neural pathways associated with various neurological, neuropsychological, and neuropathological processes.

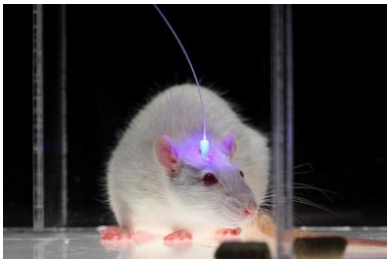


Figure 3. Optogenetics

Conventionally, the majority of in vivo optogenetics implementation relies on optical fibers connected to external lasers. Albeit simple and powerful, the optical fiber approach is limited due to its tethered operation and lack of design flexibility, which severely limits animals' behavior or even leads to entanglement as animals' movement is bounded by the wires attached to them. Mostly, they also need to be separated from their natural habitat and be confined in an experiment area because of the many machines that needed to be attached to them. This makes researchers unable to conduct complex neuroscience experiments involving social behavior, aggression, and related complex naturalistic behaviors. Moreover, relatively large modulus of optical fibers and commonly used metal makes combining these tools together challenging because it can cause large tissue damage. It can also lead to spatial-mismatch between points of light emission and neural recording, preventing precise, accurate analysis of neural activities at spatially targeted regions.

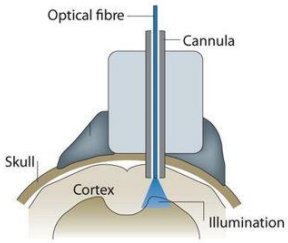


Figure 4. Separated optogenetics module using optical fibers

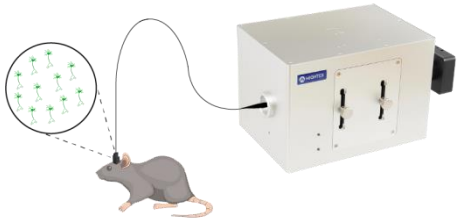


Figure 5. Tethered neural implant system

Recent advances in materials and microfabrication techniques have tried to overcome this issue by creating optical neural probes that are customizable in terms of size, dimensions, and functions for minimally invasive versatile operation. This microfabrication-based method not only allows the use of various materials (e.g., silicon, SU-8, polyimide (PI), etc.) to build highly biocompatible implants but also facilitates the direct integration of probes with micron-scale light sources and wireless control units. Together these features enable opportunities for tether-free chronic optogenetics in awake, behaving animals. This direct integration of probes with micron-scale light sources make simultaneous stimulation and neural recording with high spatial matching achievable.

Figure 6. Wireless neural implant system

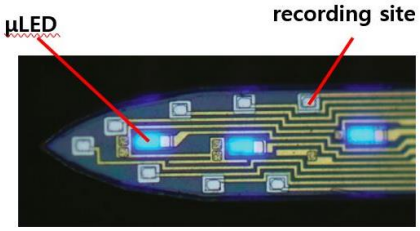


Figure 7. Neural recording electrode integrated with μLED

2. Research Objectives

By identifying the limitations of currently available technology in neural stimulation and recording, this research aspires to tackle those issues by designing a neural probe that fulfills these utilities and characteristics:

1. Thin and flexible neural probe to prevent damage to the surrounding tissues
2. Wireless neural implant system to allow more design flexibility, give more freedom for animals' movement and open up possibilities for complex neuroscience experiments involving social behavior, aggression, and related complex naturalistic behaviors

3. Simultaneous neural recording and stimulation system with high spatial matching to allow more complex and larger range of neuroscience experiments to be conducted

3. Research Methods

3.1 Fabrication of neural probes

a. Thin and flexible 3D-printed optogenetics probe (3D-POP)

For seamless chronic implantation into living subjects, neural probes should have physical properties (i.e. elastic modulus) well-matched with biological tissue. Our 3D-POPs can be fabricated as thin as 60 μm, which is comparable to the thickness of a human hair, to enhance their mechanical compliance for biomechanical compatibility with soft brain tissue. Another advantage of this 3D-POP is that it does not require time-consuming microfabrication procedures and expensive materials and cleanroom machines. It is also highly customizable and can be made with minimal skills, equipment, and training, thus significantly reducing the overall cost, time, and effort required for the construction of neural probes.

b. Probe with transparent substrate

We chose the method of stacking probe with μ-ILEDs and probe with electrodes for simultaneous photostimulation and electrophysiological recording. So the transparent, flexible, thin (6 μm) polyethylene terephthalate (PET) was set up as the substrate of the probe for the electrode. Two kinds of electrodes were fabricated. Gold and graphene were adopted as materials for electrodes, respectively. In the electrophysiological recording, gold is a very common electrode material, and the results using it have been reported a lot. However, it is opaque that light emitted from μ-ILED cannot pass through the electrode and induce spatial mismatches of photostimulation and neuronal signal recording. In contrast, graphene, which is a transparent material, has nice electrical conductivity, can be

perfectly aligned with the path of the light. These probes can be fabricated through the microfabrication process in cleanroom.

c. Structure and steps of fabrication

The bifunctional neural probe consists of optogenetic probe and neural recording probe.

Steps of fabrication of 3D-printed optogenetic probe:

1. With an appropriate computer-aided design (CAD) file loaded in a 3D printer (B9 Core 530, B9 Creations), the desired shapes and dimensions of probe substrates are printed with a photopolymer (B9R-4-Yellow Resin, B9 Creations)
2. Silver paste (P-100, CANS) is spread onto the patterned probe substrates with a rubber blade to render the electrodes into the microgrooves.
3. Soldering of μ-ILEDs (220 μm × 270 μm; TR2227, Cree Inc.) at the tips of probes
4. Coat shanks with a polydimethylsiloxane (PDMS; 1 μm)/parylene C (6 μm) bilayer to provide biocompatibility, chemical inertness, and waterproofing (vapor permeability of parylene C = 0.083 g mm m^{-2} day^{-1})

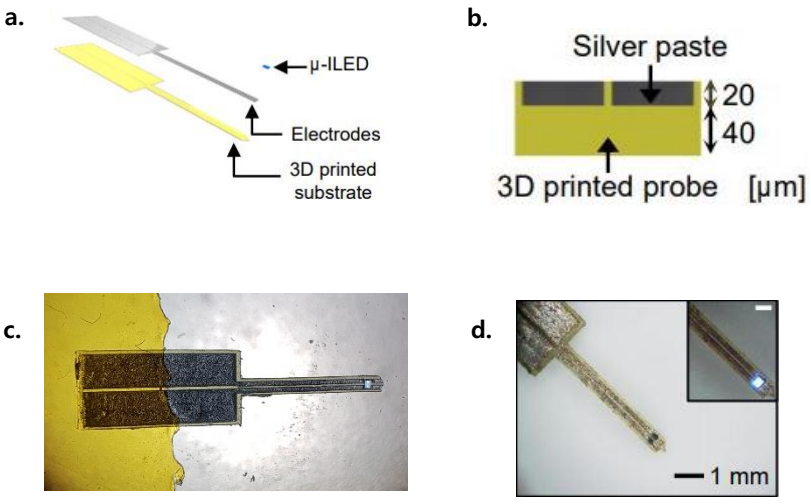


Figure 8. 3D-printed optogenetic probe (a) Exploded view illustration of the probe illustrating its basic architecture (b) cross-section of the probe (c) Photo of the probe (c) μ-ILED lights up

Steps of fabrication of gold neural recording probe:

1. Deposit Au/Cr (150 nm / 5 nm) on PET substrate.
2. Pattern electrodes and signal tracing lines via photolithography and wet etching.
3. Encapsulate probe with SU-8 epoxy (6 μm) except electrodes.

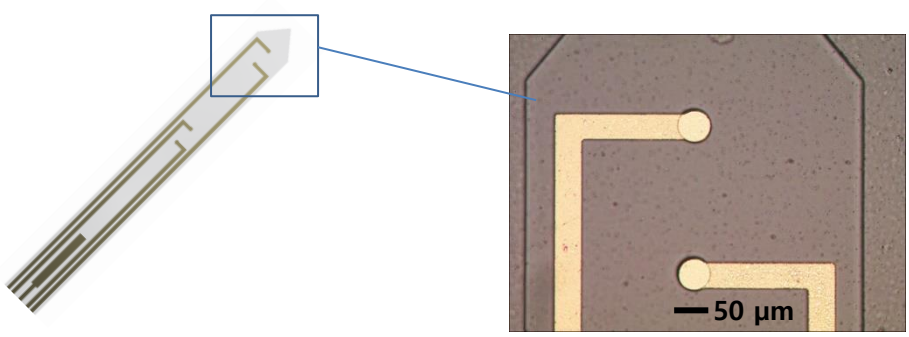


Figure 9. Gold neural recording probe

Steps of fabrication of graphene neural recording probe:

1. Deposit Au/Cr (150 nm / 5 nm) on PET substrate.
2. Pattern signal tracing lines via photolithography and remove useless metal with etchant.
3. Use wet transfer to move graphene onto the PET.
4. To enhance electrical characteristics, spin coat conductive polymer (PEDOT:PSS).
5. Pattern graphene electrodes by photolithography and oxygen plasma etching.
6. Encapsulate probe with SU-8 epoxy (6 μm) except electrodes.

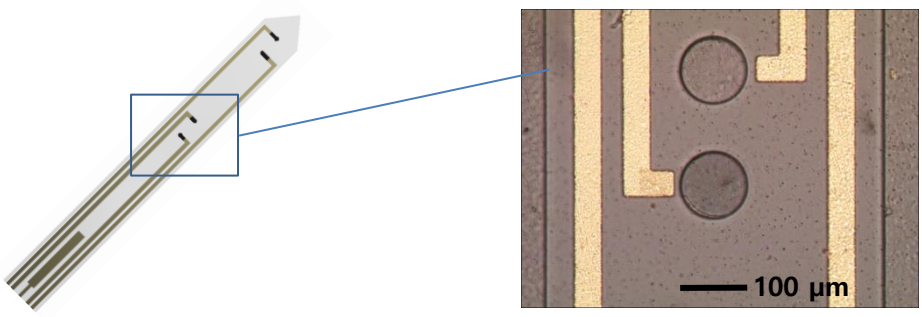


Figure 10. Graphene neural recording probe

3.2 Design of wireless neural recording system

Bluetooth Low Energy (BLE) technology is chosen to enable wireless connection between PC and neural implant. BLE allows easy long-range access to a specific device among many available devices in the vicinity (10–100 m). BLE wireless controls have several advantages for neuroscience experiments over other wireless technologies, such as infrared (IR) and radiofrequency (RF). Unlike IR or other RF systems, BLE provides orientation-independent wireless control, thus guaranteeing reliable omnidirectional activation of a target device. This feature ensures the stable operation of wireless devices in freely moving animals. In addition, unlike IR systems where obstacles can completely incapacitate the wireless control, BLE offers high penetration power of signals through various materials like wood, glass, plastic, and walls.

In BLE communication, there are two parts of devices: Master/Central and Slave/Peripheral. Master/Central BLE device initiates an outgoing connection request to an advertising peripheral device. Slave/Peripheral BLE device accepts an incoming connection request after advertising. In this research, the neural implants will be the Slave/Peripheral BLE device, it will start advertising brain neural signals once it accepts a connection request from the Master/Central BLE device. Even though the neural implant might receive signals from PC, we will address it as 'transmitter' because most of the time it will transmit brain signals. PC may send signals to control the neural implant but most times it receives brain signal from it, therefore we will address it as 'receiver'. A Master/Central BLE device needs to be connected to the PC for the PC to receives the transmitted signal through its serial port. When PC wants to start accepting or processing the neural data, it will send a connection request through the Master/Central BLE device to the neural implant that acts as the Slave/Peripheral BLE device.

a. Neural Implant - Slave/Peripheral BLE Device (Transmitter)

RHA2116 is an integrated circuit (IC) from Intan Technologies for neural recording which already includes filter and amplifier inside. This IC will be connected to the recording electrodes to receive neuron signals from them. BLE module(Taiyo yuden EYSHSNZWZ) will be used as the Slave/Peripheral BLE Device and this BLE

module will be connected to IC to control the IC and receive neuron signals data from it. BLE module will also be connected to the μ-ILEDs on the neural probe to control them. All BLE devices have their own universally unique identifiers (UUIDs). Here, we modified UUID of this BLE module to make our neural device advertises specific UUID that we can control. For the power supply, Lithium ion (Li-ion) battery is used alongside with Low dropout (LDO) voltage regulator.

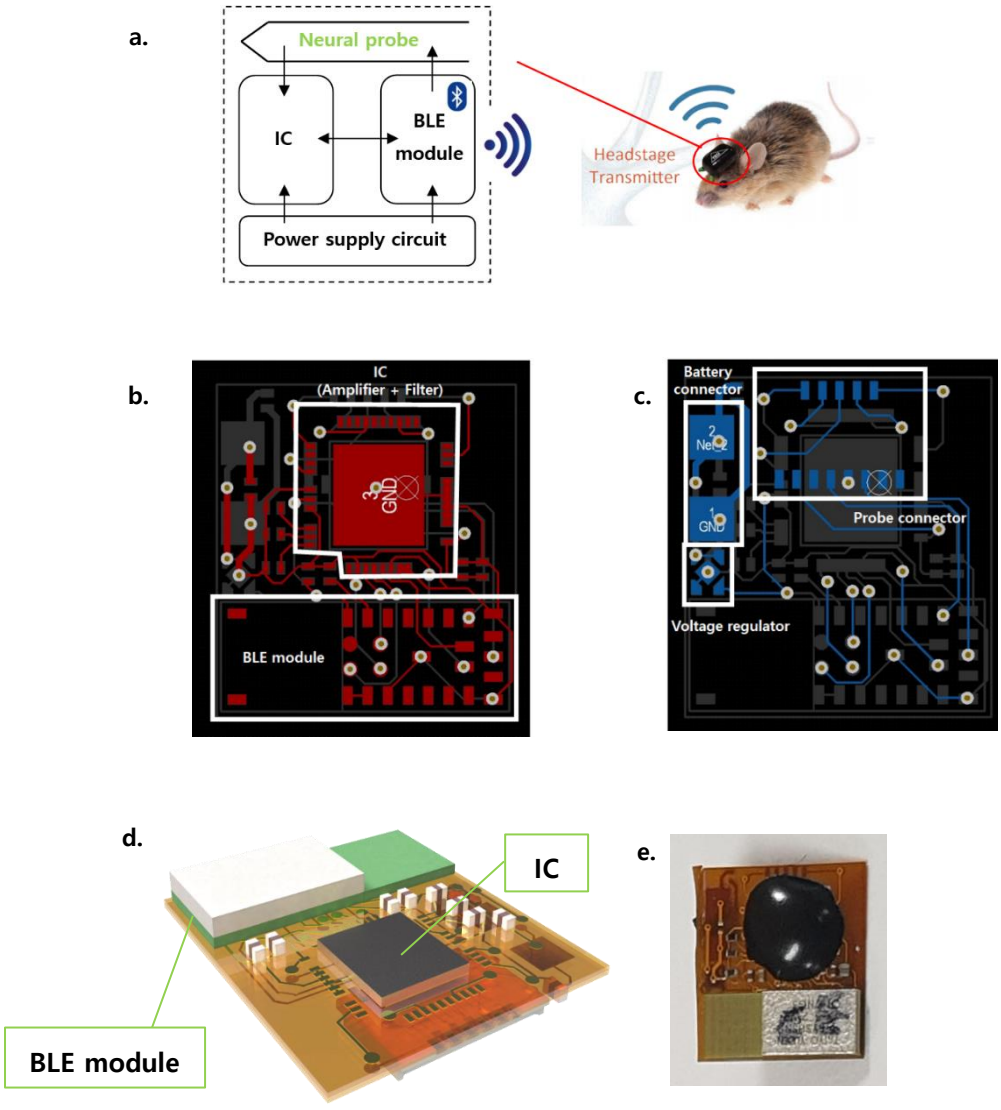


Figure 11. BLE wireless control module design. (a) Schematic diagram of a BLE wireless control module (b,c) Printed circuit board (PCB) layout of the diagram in (a), showing the top and bottom layers, respectively (d) 3D layout of printed circuit board (PCB) (e) Photo of PCB

In this research, experiment with animal cannot be conducted, therefore our user-interface cannot be tested directly using our neural implant. For user-interface testing purpose, nRF52832 PCA10040 Development Kits will be used as the transmitter instead. This device has the same architecture as Taiyo Yuden: BLE module that we used as Peripheral BLE Device in the neural implant.

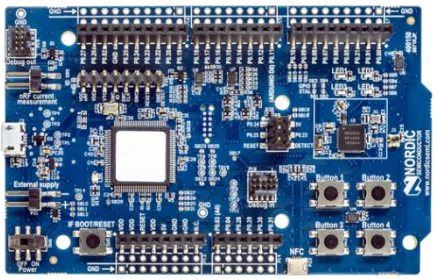


Figure 12. nRF52832 Development Kits as peripheral BLE Device

To program 'nRF52' PCA10040, SEGGER embedded studio is used and example code from 'nRF52' SDK is modified to fit our usage.

a.

```
* This function setup the next buffer in queue and print the completed buffer.
*/
void saadc_callback(nrf_drv_saadc_evt_t const * p_event)
{
        if (p_event->type == NRF_DRV_SAADC_EVT_DONE)
        {
                ret_code_t err_code;
                buffer_number ^= 0x01;
890             saadc_event_callback = true;
                err_code = nrf_drv_saadc_buffer_convert(p_event->data.done.p_buffer, SAMPLES_IN_BUFFER-2); //sizeof(p_buffer):4
                //NRF_LOG_INFO("a: %d", sizeof(p_event->data.done.p_buffer));
                p_event->data.done.p_buffer[SAMPLES_IN_BUFFER-2]=1023;

                /*for (int i=0; i<SAMPLES_IN_BUFFER; i++){
                p_event->data.done.p_buffer[i]=i;
                }*/

                APP_ERROR_CHECK(err_code);
900
        }
}
```

b.

```
// Enter main loop.
for (;;)
{
        if (saadc_event_callback) {
                ble_fs_send_adc_data(m_conn_handle, &m_ble_fs, &(adc_buffer.m_buffer_ble[buffer_number * adc_ble_notify_size]), adc_ble_notify_size);
                //ble_fs_send_adc_data(m_conn_handle, &m_ble_fs, &(adc_buffer.m_buffer_ble[(buffer_number * adc_ble_notify_size) + (adc_ble_notify_siz
                saadc_event_callback = false;

        }
        idle_state_handle();
}
```

c.

```
/**@brief Function for handling write events to the LED characteristic.
 *
 * @param[in] p_ble_fs     Instance of LED Button Service to which the write applies.
 * @param[in] cmd          USER Interactive cmd.
 */
static void ui_write_handler(uint16_t conn_handle, ble_fs_t * p_ble_fs, uint8_t* p_data, uint8_t len)
{
940     ret_code_t err_code;
        uint8_t cmd = p_data[0];

        //NRF_LOG_INFO("%d\r\n", p_data[0]);

        switch (cmd)
        {
        case 0x31: // start ADC data, when send 1
        {
                nrf_delay_ms(1000);
950             //NRF_LOG_INFO("starting ble fast with adc\r\n");
                start_mux_input_selection();
                saadc_sampling_event_enable();
                enable_timers();
        }
        break;

        case 0x34: // set PWM: LED1, 10Hz, 10ms duty
        {
                        if (pwm1_frequency == FREQ2) {
                                deinit_ble_pwm1();
                        }
                        else{
                        if (ble_pwm1_enable_flag) {
                                deinit_ble_pwm1();
                        }
                        init_ble_pwm1(PWM_OUTPUT1, FREQ2, DUTY2);
                        }
        }
        break;
```

Figure 13. Fast_adc_with_ble sample project with modification in main.c
(a) saadc_callback: data format of ADC buffer, 121 samples per array buffer sent at once
(b) main loop (if(saadc_event_callback))
when transmitter receives 0x31, saadc_event_callback will change to True, gets in if loop and send data, change state back to False and repeat
(c) static void ui_write_handler: transmitter do some action based on command sent by receiver

b. PC – Master/Central BLE Device (Receiver)

For the PC to be able to receive the data transmitted by the neural device through BLE, a Master/Central BLE Device which acts as a 'receiver' needs to be programmed. Previously, we set our peripheral device (neural implant) to advertise a specific UUID. Before 'receiver' can get the neural data, it needs to connect to our neural implant by scanning for available peripheral devices and select to connect to that specific UUID which we set for our neural implant to advertise. For the neural implant to start advertising the neural signal it gets from the brain, the Master/ Central device needs to send a connection request to the neural implant.

After receiving the neural signal, this Master/Central device will pass the data to the PC's serial port. The user interface will then extract data from the serial port to be plotted and processed further.

In this research, a high rate of data transmission is needed to keep up to the fast-incoming brain signals from the neural implant. Neural implant (Slave/Peripheral device) sends data with a speed of 1.2Mbps roughly. To print out correct and complete data, the Master/Central device needs to be able to keep up with this rate. Many types of communication protocols can be used to bridge the connection between receiver with transmitter, such as Universal Asynchronous Reception and Transmission (UART) and Universal Serial Bus (USB). We first tried to use the UART protocols as it is simpler than USB's protocol. However, the data received seems to be corrupted or not complete because it cannot keep up with the peripheral device's transmitting speed. Therefore, we decided to use USB communication because it offers high data rates. 'nRF52840' PCA10056 Development Kits is used as a central device because it supports 'USB communication'.

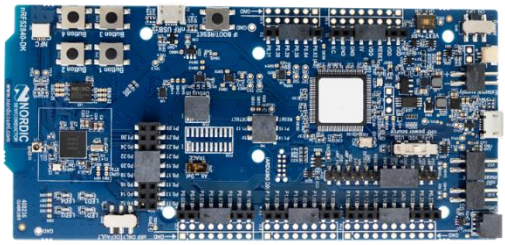


Figure 14. nRF52840 Development Kits as central BLE Device

To program 'nRF52840', SEGGER embedded studio is used and example code from 'nRF52840' SDK is modified to fit our usage.

```
290 static void nus_data_handler(uint8_t * p_data, uint16_t data_len) //check
291 {
292         bsp_board_led_invert(LED_BLE_NUS_RX);
293         //NRF_LOG_RAW_INFO("Received data from BLE NUS. Writing data on CDC ACM.\r\n");
294         //NRF_LOG_HEXDUMP_DEBUG(p_data, data_len);  //data_len = 243
295
296         memcpy(m_nus_data_array, p_data, data_len);  //sizeof(uint8_t)=1
297
298         uint16_t j = 0;
299         uint8_t a,b,c,d,k,t;
300         uint16_t l;
301         for (int i = 0; i < data_len - 1; i = i+2){
302          k = m_nus_data_array[i];
303          t = m_nus_data_array[i+1];
304          l = 16 * 16 * t + k;
305
306          a = l / 1000;
307          b = (l - 1000 * a) / 100;
308          c = (l - 1000 * a - 100 * b) / 10;
309          d = l % 10;
310          number_array[j] = a + 48;
311          number_array[j+1] = b + 48;
312          number_array[j+2] = c + 48;
313          number_array[j+3] = d + 48;
314          number_array[j+4] = 45;
315          j = j + 5;
316         }
317         number_array[j-1]=13; //\r
318         number_array[j]=10; //\n
319         j++;
320
321         app_usbd_cdc_acm_write(&m_app_cdc_acm, number_array, j);
```

Figure 15. usbd_ble_uart_pca10056_s140 sample project with modification in main.c nus_data_handler: print out the received 121 decimal numbers to PC's serial port

3.3 Software development (user interface)

Currently, there exist applications for acquiring and visualizing data received from neural probe. One of the examples of a well-known application is OpenEphys (https://open-ephys.org/).

However, each of these applications is only compatible with specific equipment which is expensive and does not provide simultaneous recording and stimulation. These types of equipment and software also do not support wireless connection. Therefore, we need to develop our own user interface to support visualizing data acquired from wireless neural recording and simultaneously allow users to control the μLED on the neural probe to stimulate brain cells.

Python is used to develop this user interface as it is simple to program in this language while still offer high quality of wireless real-time plotting. Below are the libraries and class used in the development of the user interface:

pySerial (https://pypi.org/project/pyserial/)

pySerial is a python library that provides access to the PC's serial port. Port's name and appropriate baud rate need to be specified.

PyQtGraph (http://www.pyqtgraph.org/)

PyQtGraph is an open source python library which supports interactive 2D plotting. It is built on PyQt4 / PySide and numpy and is intended to be used in mathematics / scientific / engineering applications. Despite being written entirely in python, the library is very fast due to its heavy leverage of numpy for number crunching and Qt's GraphicsView framework for fast display.

Compared to other 2D plotting library such as matplotlib, PyQtGraph offers faster plotting-speed. Our interface needs high plotting speed to keep up with the high incoming data rates. Although Matplotlib has better graphics than PyQtGraph, Matplotlib's plotting speed is not enough to keep up with the high incoming data rates. Therefore, PyQtGraph is chosen to support our real-time data visualization as it provides rapid plot updates and real-time interactivity.

PyQt5 (https://pypi.org/project/PyQt5/)

PyQt is a library that allows us to use Qt graphical user interface (GUI) framework from Python. Qt is a free and open-source widget toolkit for creating graphical user interfaces as well as cross-platform applications. Qt itself is written in C++. By using it from Python, applications can be built much more quickly while not sacrificing much of the speed of C++. PyQt5 will be used to build the GUI basic layout and widget such as buttons and display boxes.

QThread (https://doc.qt.io/qt-5/qthread.html)

QThread is a class that is included inside QtCore, which is a module that comes along when PyQt5 is installed. QThread class provides a platform-independent way to manage threads. Our interface needs to read data from serial port and the data need to be processed before it can be fed into the plotting system. Because the neural signal data comes in with really fast speed (roughly 1.2Mbps), these tasks are really heavy to be run only by one thread, which results in slow plotting speed which cause the plotted data to lag behind the actual data. It may also cause the interface to be unresponsive when the plotting starts, which also means the buttons for controlling the μ-ILEDs on the neural probe become

unresponsive.

To be able to tackle this issue, threading is needed to split the tasks into two simultaneously (or pseudo-simultaneously) running tasks. The first thread works on acquiring and processing incoming data while the second thread works on plotting the data and sending signals to neural probe. After this division of work, the load of each thread becomes lighter and it results in faster plotting speed and responsive interface even when the plotting is running. By using threading, simultaneous real-time data plotting and neural probe controlling are achievable.

The performance of the plotting should be constant throughout the simulation. If the interface keeps receiving data and buffer is filled up, it causes the plotting speed to decrease over time. Therefore, the buffer needs to be flushed and replaced with new incoming data regularly to prevent the filled-up buffer from hindering the performance of the plotting. The design of the user interface is also made as simple as possible because stylish design requires rendering of image which will make the program's load heavier and therefore cause a decrease in the plotting speed.

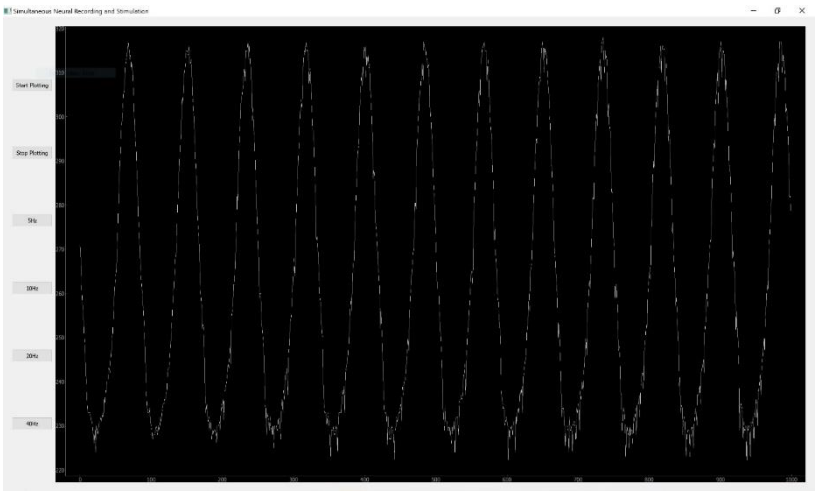


Figure 16. User interface built with python
-Start/Stop plotting button is for plotting the received neural signal
-5Hz, 10Hz, 20Hz, 40Hz is for stimulating the μLED on the neural probe to blink according to the specified frequency

3. Results

Validation of fabricated probe

a. Optogenetic probe performance test

3D printing techniques based optogenetic probe was successfully fabricated. A test was run to validate the functionality of the probe. By applying voltage to the fabricated optogenetic probe, we can measure the resulted current and optical intensity value of the probe to see whether its characteristic is sufficient to perform optogenetics. From the measurement, the current and optical intensity are plotted in the graph below. Our probe will be operated at 3V and from the graph, we can see that at 3V our probe managed to produce around 80mV/mm$^{2.}$ Optical intensity above 1mV/ mm^2 is enough to carry out optogenetics, this validates that the probe we fabricated is able to produce optogenetic stimulation well.

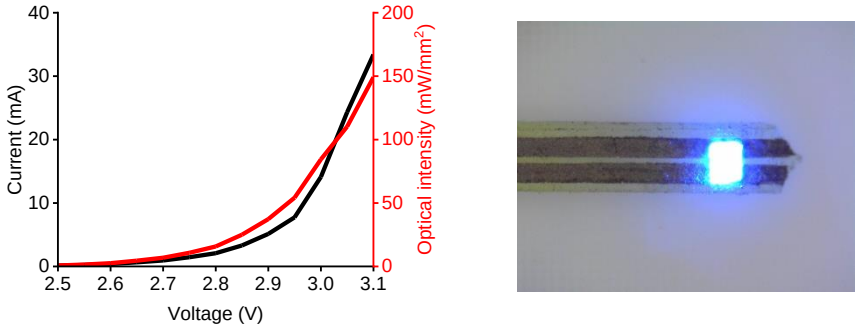


Figure 17. (a) Voltage vs current/optical intensity graph of our 3D-printed optogenetics probe (b) the attached μILED successfully lights up

b. Impedance measurement of probe's electrodes

3-electrode method is used to measure the impedance of the probe we fabricate. Probes (working electrode), counter electrode (Platinum), and reference electrode (Ag/AgCl) are put in 0.9 % saline solution and the impedance is measured through LCR meter.

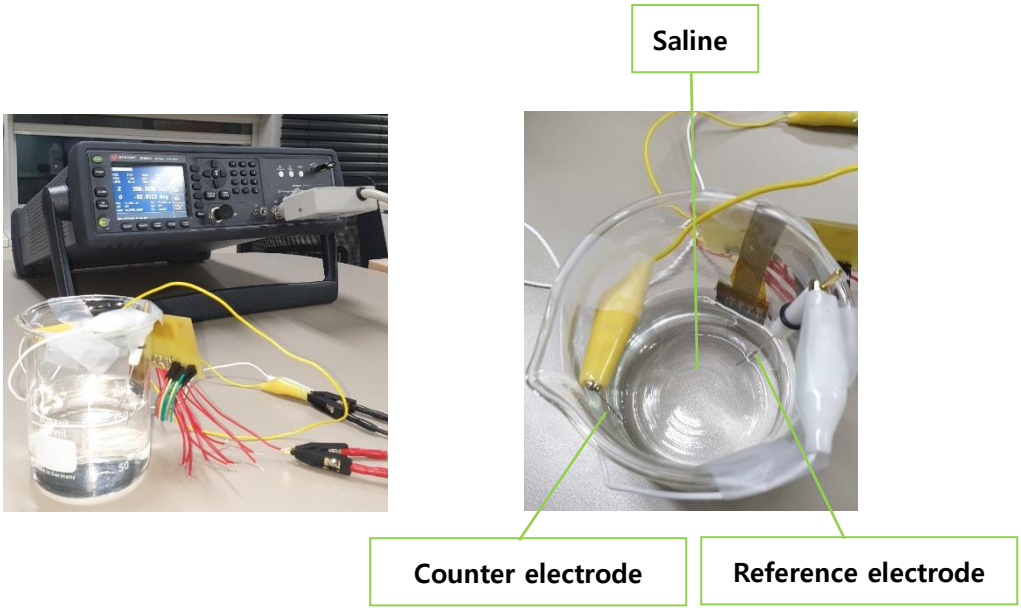


Figure 18. Set up of probe's electrodes' impedance measurement using 3-electrode method

From the measurement, the impedance value versus frequency graph below is plotted for gold electrode and graphene electrode respectively. The impedance of electrode is usually compared at the state of 1kHz. The resulted graph shows that graphene electrode has better impedance than gold electrode. Keep in mind that in the fabrication method, graphene electrode is coated with conductive polymer (PEDOT:PSS) to enhance its electrical characteristics.

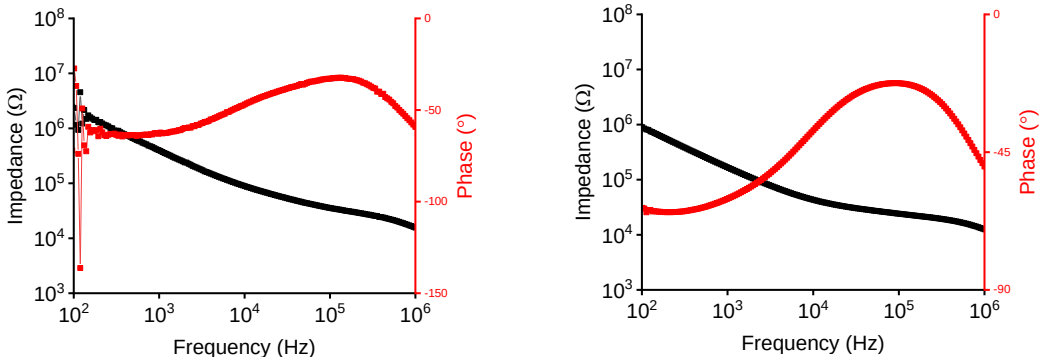


Figure 19. Impedance/phase vs frequency graph of (a) gold (*Φ = 50* µm) electrode (b) graphene (*Φ = 100* µm) electrode

BLE wireless control module circuit validation

Brain neural signal such as EEG has a feeble amplitude and there will be a significant presence of electrical noise at low frequencies in most environments caused by other's movement artifacts. Therefore, proper amplification to enhance the signal strength up to a level that permits the working of ADC (Analog to Digital Converter) at its effective resolution and noise removal is a must in pre-processing the neural signal before it gets transmitted. This means that the BLE wireless control module circuit needs to be able to act as a filter and amplifier. The resulted bode plot shows that the circuit works well as a filter with 3-dB cutoff frequency at 10Hz and 500Hz and as an amplifier with gain 200 (46dB).

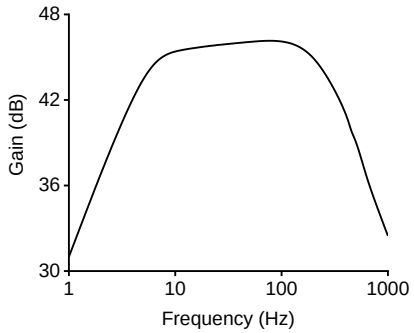


Figure 20. Bode plot of the circuit: 3-dB cutoff frequency at 10 Hz, 500 Hz and Gain = 200 (46dB)

ADC (Analog to Digital Converter) calibration

The neural signals extracted from the brain are analog signals, therefore it needs to be transformed into digital signals before they can be transmitted wirelessly and plotted by the user interface. Peripheral BLE Device will be in charge of being the ADC and signal will be processed before they transmit it over to the central BLE device. In the neural implant circuit, we need to design/program ADC function into BLE module. The programmed ADC function can convert analog data in the range of 0-2.4V with 10-bit resolution. To ensure and test that the programmed ADC functions correctly, calibration is needed. A well-working ADC function should show constant proportionality between sampled ADC value and real voltage value. To check this relationship, we plotted the converted value

resulted from the ADC function vs the real voltage value that we inputted. The range of input voltage used is from 0.235 to 2.235 V because the output of IC on our customized chip is in that range.

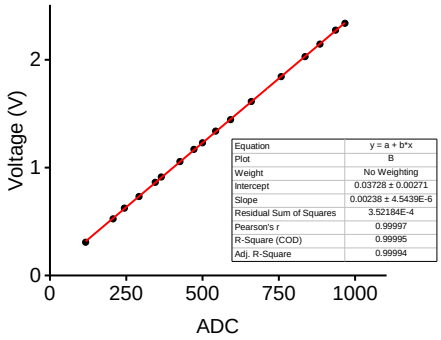


Figure 21. ADC calibration

The graph resulted from the calibration shows linearity, which means the ADC functions correctly.

BLE Peripheral Test (Taiyo yuden BLE Module/ nRF52382)

BLE communication function is added into nRF52832 and tested to check if the peripheral device can sample input data and send it to central successfully. nRF52832 converted analog data into digital, and send that data to central BLE. In this test, wave generator will be the source of the input data. At the time of this test, real-time plotting system on PC was not developed yet so we used a smartphone app: nRF connect (made by manufacturer of nRF chip) as the central BLE device. nRF connect app saves data in hexadecimal number, so the data need to be converted to decimal before it is plotted. Matlab is used to do this transformation.

For testing, we set BLE Peripheral to sample data for 5ksample/s, which is the range of EMG signal rate because in later stage we will use EMG signal as real signal source. We tested with 100 Hz and 500 Hz sine wave and the resulted graph is satisfactory.

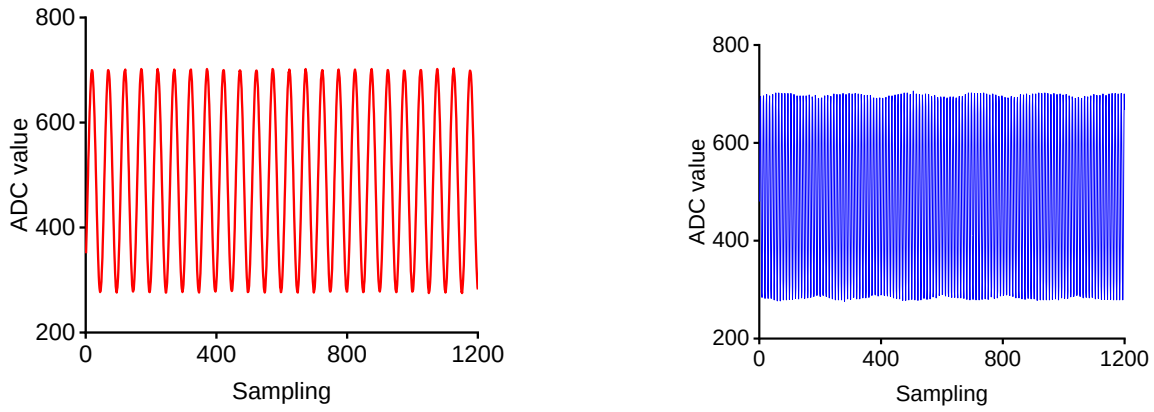


Figure 22. Plot of data received by central when peripheral sample 100Hz sine wave(left) and 500Hz(right) and send to central

Real signal testing using EMG signal

After peripheral BLE is tested with an input signal from a wave generator and it shows good result, the next step is to test it with real signal. EMG(Electromyography) signal; signal produced by motor neurons; is chosen as the source of our real signal because we cannot test brain signals as animal experiments cannot be conducted in this research. Data is collected using a smartphone app: nRF connect (smartphone works as central BLE device). The circuit tested include IC from Intan Technologies and BLE module (Taiyo yuden EYSHSNZWZ). Arm's motor neuron will be used as input. Using commercial gel-electrode, 2 bipolar inputs are placed on the arm and 1 ground is placed on the elbow. Electrodes are connected to the circuit by wire. The produced EMG data is transmitted to a smartphone using nRF connect app. The data is then processed using Matlab and the graph below is resulted.

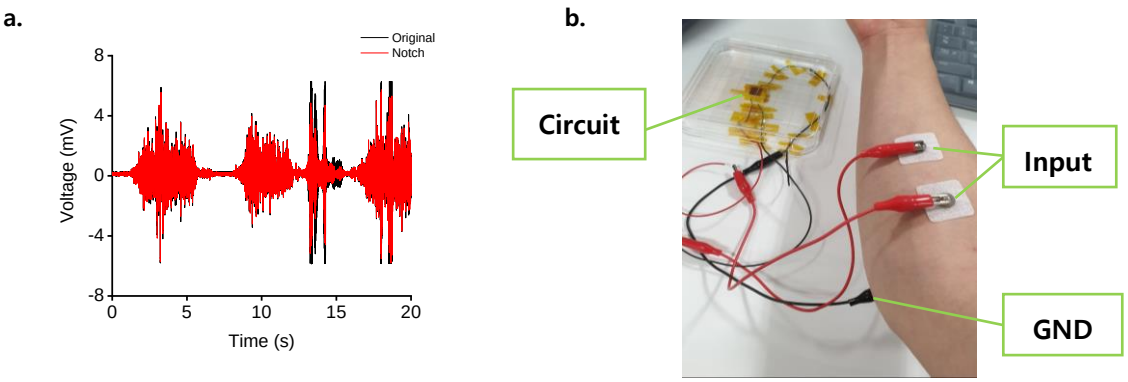


Figure 23. (a) Plot of data received by central: black: original signal, red: applied 60 Hz notch filter using Matlab (b) EMG test setup

User Interface Testing

To evaluate the performance of the device, the wave function generator is used to generate a wave signal which acts like a neuron signal received from the neural probe. Peripheral Bluetooth module/transmitter (nRF52832) is powered by a 3V DC power supply and connected to the wave generator to receive signal input. This signal is transmitted to the central Bluetooth module/receiver (nRF52840) wirelessly and receiver passes on the signal to PC's serial port which then is plotted by the user interface. The equation of the generated wave signal is controlled, which means we can check the correctness of the data plotted by the user interface. To test the performance of UI in controlling the μ-ILEDs, the neural probe is connected to peripheral BLE's output and we can test to wirelessly turn on/off the μ-ILEDs that are attached on the neural probe by clicking on the 5Hz, 10Hz, 20Hz or 40Hz button on the user interface. This test can be done simultaneously while data plotting continues to run.

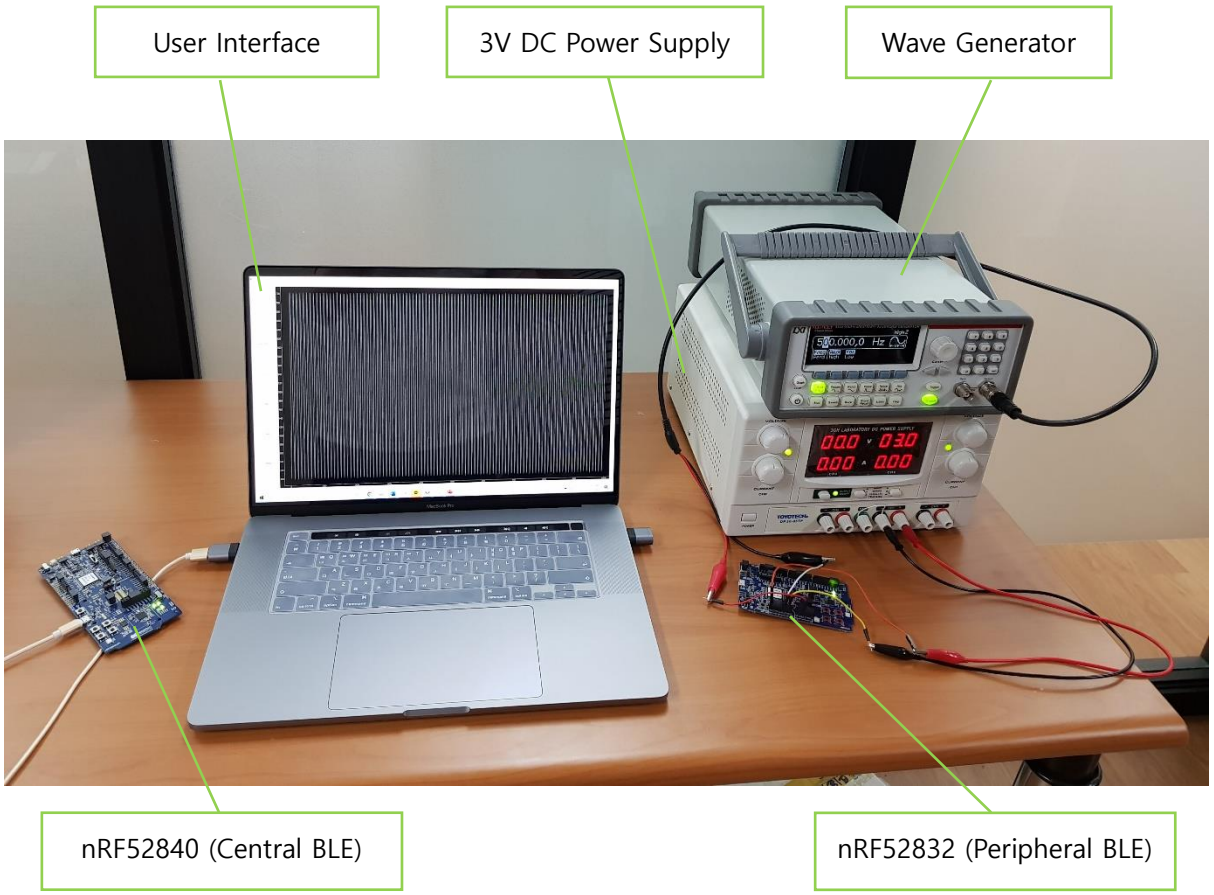


Figure 24. Set up for user interface's plotting performance

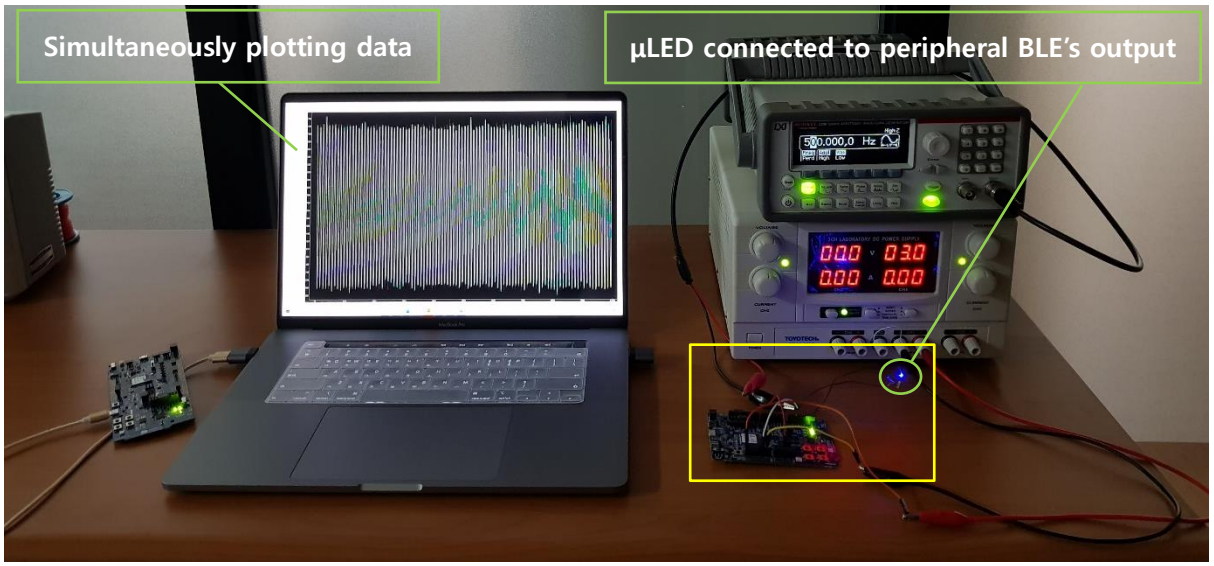
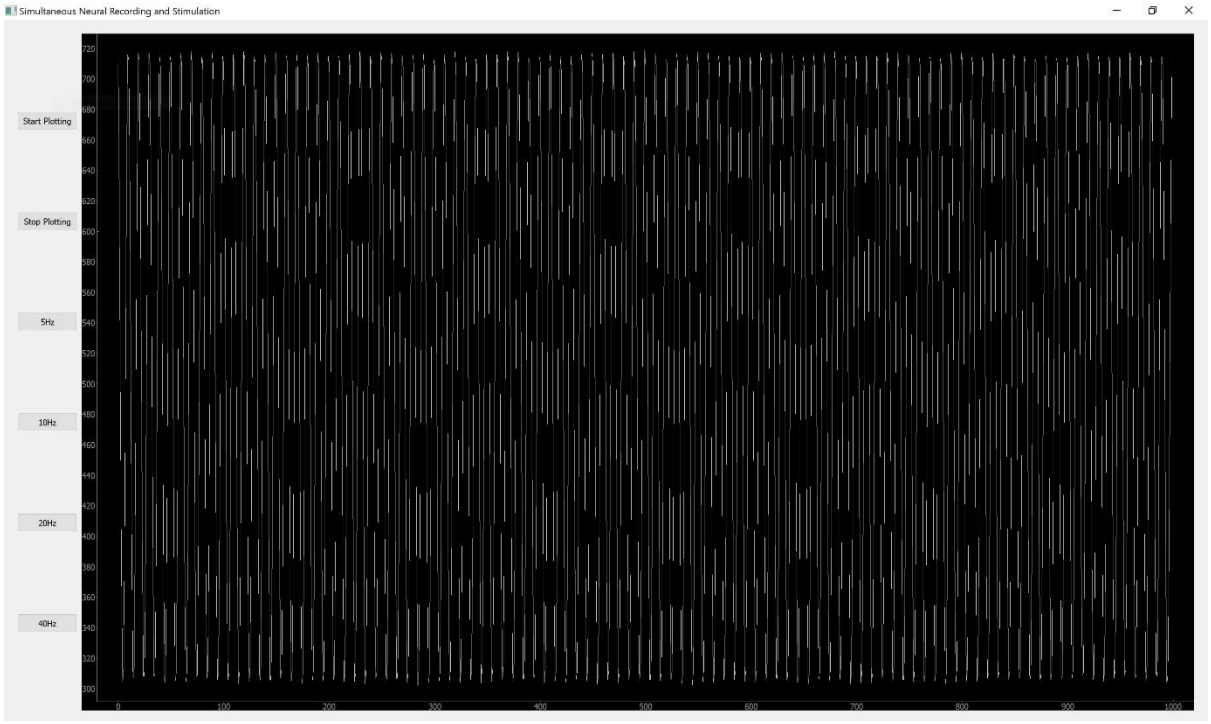


Figure 25. Set up for user interface's µLED controlling test



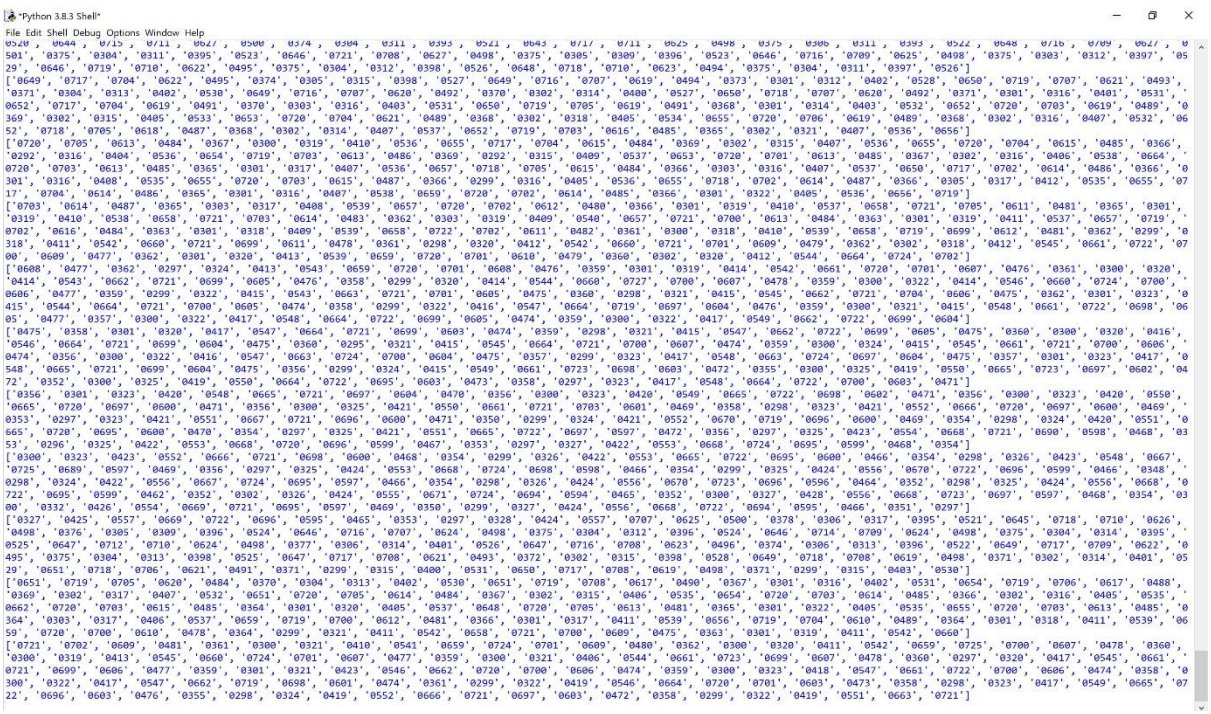
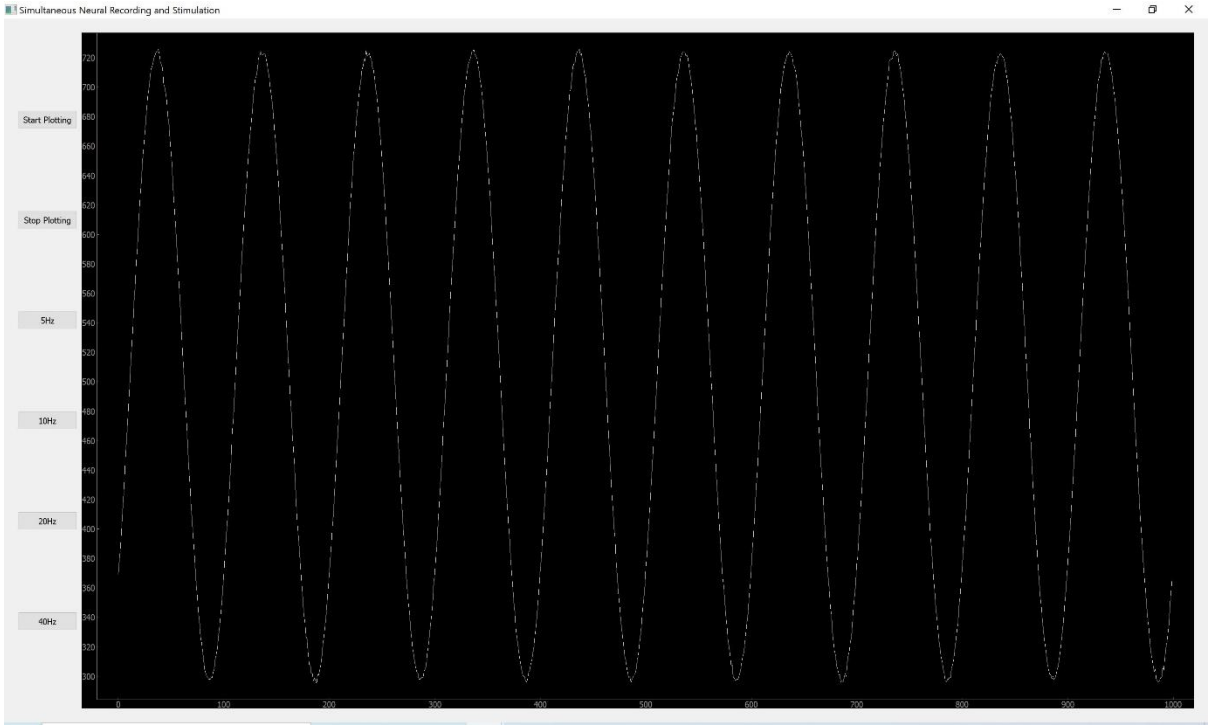


Figure 26. Plotting result and data of 500Hz sine wave received from wave generator wirelessly



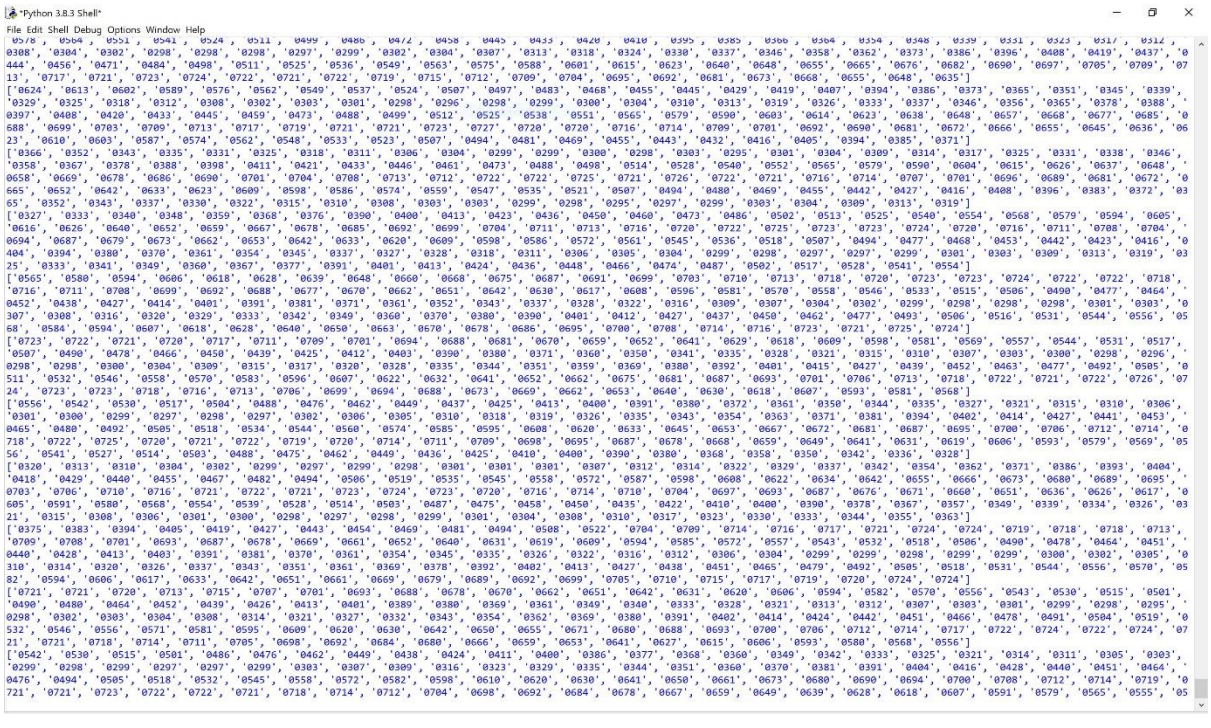


Figure 27. Plotting result and data of 50Hz sine wave received from wave generator wirelessly

4. Conclusion and Future Research Plan

By identifying the limitations of currently available technology in neural stimulation and recording, this research is conducted in the hope to tackle each of the issues found. Most conventional neural probes are still made of rigid material which might cause damage to surrounding tissues. Brain research experiment where simultaneous recording and stimulation is needed is not feasible yet as existing technology has not been able to support that ability. Wires are still used by most recording tools and this tethered operation significantly limits animals' free behavior and movement. This prevents complex neuroscience experiments involving social behavior, aggression, and related complex naturalistic behaviors.

For our neural probe, 3D-printed based fabrication method is chosen to provide highly customizable thin and flexible neural probe with low-cost, time, and effort. To allow simultaneous recording and simulation, μ-ILEDs are integrated into the 3D printed probe which enables the neural probe to perform optogenetics stimulation while simultaneously still recording the neural signal. For implementing wireless connection, BLE is chosen as it provides easy long-range access to a specific device, ensures stable operation within the required range, and offer high-penetration power through obstacles. To set up the BLE connection between PC and neural implant, nRF52832 is connected to the circuit in neural implant and used as a peripheral BLE device while nRF52840 is used as a master BLE device and connected to PC. nRF52840 will scan, connect, and send a request to nRF52832. Upon receiving this request, nRF52832 will start to advertise received brain signal to nRF52840 which then is passed on to PC's serial port. Both of them are programmed with SEGGER embedded studio to achieve this functionality. To be able to plot the received brain signal data and control the μ-ILED wirelessly, a user interface is programmed with python using pyqtgraph as the plotting library and pyQt as the GUI library.

The 3D printed optogenetics probe, BLE wireless connection system, and user interface produced in this research are able to achieve real-time simultaneous recording, monitoring, and stimulation. However, currently, our user interface has not yet been able to save the incoming brain signals and does not support analysis

of brain signals. In future works, the user interface is envisioned to be able to save data into a format that is compatible with existing neural signal analysis tools (e.g: Open Ephys) so that useful information such as spikes can be extracted from the brain signal.

5. Reference Literature

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[5] Hasegawa, T. et al. A wireless neural recording system with a precision motorized microdrive for freely behaving animals. Sci. Rep. 5, 7853; DOI:10.1038/srep07853 (2015).

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