Fabrication & Validation of NIR Device for Photo Bio Modulation (PBM) Therapy on Affective Disorder

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Fabrication & Validation of NIR Device for Photo Bio Modulation (PBM) Therapy on Affective Disorder

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Requirement for the degree of

Bachelor of Technology

in

Biomedical Engineering

By

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*(Roll NO: **120BM0806**)*

Under the guidance of

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NATIONAL INSTITUTE OF TECHNOLOGY ROURKELA

Dr. Bibhukalyan Prasad Nayak

Asst. Professor

May 08, 2024

Supervisor's Certificate

This is to certify that the work presented in the dissertation entitled '***Fabrication & Validation of NIR Device for Photo Bio Modulation (PBM) Therapy on Affective Disorder***' submitted by ***Shreenandan Sahu***, Roll Number ***120BM0806***, is a record of original research conducted by him under my supervision and guidance. This work is submitted in partial fulfilment of the requirements for the degree of ***Bachelor of Technology in Biomedical Engineering.***

Neither this dissertation nor any part of it has been previously submitted for any degree or diploma to any institute or university in India or abroad

Dr. Bibhukalyan Prasad Nayak

“Dedicated to my Supervisor Dr. Bibhukalyan Prasad Nayak, my lab mates, my parents and the almighty.”

Declaration of Originality

I, Shreenandan Sahu, Roll Number 120BM0806, hereby declare that this dissertation entitled 'Fabrication & Validation of NIR Device for Photo Bio Modulation (PBM) Therapy on Affective Disorder' represents my original work conducted as an undergraduate student of NIT Rourkela. To the best of my knowledge, it contains no material previously published or written by another person, nor any material presented by me for the award of any degree or diploma of NIT Rourkela or any other institution.

Any contributions made to this research by others, with whom I have collaborated at NIT Rourkela or elsewhere, are explicitly acknowledged in the dissertation. Works of other authors cited in this dissertation have been duly acknowledged under the sections 'References' or 'Bibliography'.

I have also submitted my original research records to the scrutiny committee for evaluation of my dissertation.

I am fully aware that in case of any non-compliance detected in the future, the Senate of NIT Rourkela may withdraw the degree awarded to me based on the present dissertation.

May 08, 2024
NIT Rourkela

Shreenandan Sahu

Acknowledgement

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Abstract

Major depressive disorder (MDD) is a common condition linked to substantial disability, alongside tendencies toward suicidal thoughts and actions. Existing treatments for MDD suffer from notable shortcomings in both effectiveness and adverse effects. FDA-approved devices for MDD treatment requires a lot of in-office procedures and are primarily suited for severely affected individuals. Consequently, there is an urgent need for compact, portable, user-friendly, and effective device-based treatment for MDD. In this research project we have successfully designed, fabricated, and validated a low-powered near-infrared (NIR) device for transcranial photo biomodulation using 830 nm NIR LED technology. The developed device has 2 hardware units (head unit and control unit) and a mobile application to control and operate the device. The hardware parts were fabricated using 3D printing. The control unit, equipped with a microcontroller (ATMEGA328P) on a custom-designed PCB, incorporates rechargeable batteries for extended usability. The head unit houses 24 NIR LEDs (with a peak wavelength of 830nm) within a specially designed 3D printed casing, providing a maximum energy output of 1200mW (calculations based on datasheet). The mobile app is used to control the energy delivered by varying the light intensity, timing, and pulsation. Validation was conducted on a zebrafish animal model using our device (control unit and app only) with specially designed applicator for fish tank, demonstrating the efficacy and functionality of the device. After this successful validation on animal model, we are aiming for human trials for which the ethical clearance is awaited.

Keyword: Affective Disorders, Depression, low-level light therapy, major depressive disorder, near infrared (830nm), transcranial photo biomodulation (t-PBM).

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Chapter 1: Introduction

1.1. PBM and Affective Disorders

The document focuses on developing a Photo Biomodulation Therapy (PBMT) device using 830 nm infrared (IR) light wavelengths. PBMT is a non-invasive method promising to reduce the symptoms of widespread mental health conditions like depression and stress. These disorders affect millions globally, leading to a decline in quality of life and increasing healthcare costs. Major depressive disorder (MDD) affects about 16.2% of the world population, with around 4.5% in India, according to the 2015-2016 National Mental Health Survey. Current antidepressant treatments often have side effects and are only partially effective, leading to frequent relapses. Neuromodulation strategies like electroconvulsive therapy (ECT) and transcranial magnetic stimulation (rTMS) offer alternatives, but they can be complex and inconvenient. There's a clear need for a safe, effective, and user-friendly neuromodulation strategy.

Transcranial photobiomodulation (t-PBM) is a novel form of neuromodulation based on exposure to light at specific wavelengths. t-PBM with near-infrared radiation (NIR) has yielded promising early results for neuropsychiatric disorders, with a low cost and good safety profile, making it easy for self-administration. PBMT uses red or near-infrared light to help damaged tissues heal and protect themselves. By targeting specific light wavelengths, particularly 830 nm IR light, PBMT counters inhibitory nitric oxide effects, restoring cellular function. This approach offers a non-drug, non-invasive alternative for psychiatric disorders.

The project roadmap involves meticulous device design, prioritizing safety, and treatment optimization. Stringent validation includes in vivo studies (animal models and human trials). Emphasis is placed on automation for user-friendly operation and broader accessibility. The 830 nm IR PBMT device aims to revolutionize mental health treatment by specifically addressing affective disorders with light therapy. Its impact would significantly enhance the quality of life for individuals with depression.

The project seeks to contribute to advancements in mental health treatment while also humbly aiming to play a role in the broader field of medical technology, by exploring new frontiers in non-invasive therapeutic interventions.

1.2. Aim and Objectives

- Design and Fabrication of (830nm) NIR Device for Photo-Biomodulation (PBM) therapy.
- Validation of the effect of monochromatic (830nm) NIR device in counter measuring Affective Disorder in animal model (Zebra Fish)
- Comparison of effects of continuous PBM versus pulsatile PBM in treatment

Chapter 2: Literature Review

2.1. Understanding Brain Disorders

Brain disorders encompass a spectrum of conditions ranging from traumatic and degenerative disorders to psychiatric ailments. Traumatic disorders, such as stroke or brain injury, involve sudden damage to the brain, while degenerative disorders like Alzheimer's and Parkinson's manifest as gradual, progressive deterioration. Among psychiatric disorders, two prevalent conditions—bipolar disorder (BD) and Major Depressive Disorder (MDD)—highlight the complexities associated with mood-related disorders. (Merikangas, 2011) (Naeser, 2014)

Bipolar Disorder (BD) is characterized by extreme mood swings, oscillating between episodes of depression and mania. The biological underpinnings of BD involve a combination of genetic, neurochemical, hormonal, and environmental factors. Effective treatments include pharmacological approaches, such as antidepressants, and non-pharmacological interventions like behavioural therapy. (Merikangas, 2011)

Major Depressive Disorder (MDD) stands out as a widespread mood disorder, affecting approximately 12% of males and 20% of females globally. Individuals with MDD experience severe feelings of sadness, loss of interest, instability, and, in severe cases, suicidal ideation. The ethology of MDD is multifaceted, involving genetic, biological, environmental, and psychological factors. Treatment modalities encompass pharmacological interventions, such as antidepressants, and non-pharmacological options like behavioural therapy. In severe cases, Electroconvulsive Therapy (ECT) may be considered. (Association, 2013)

2.2. Understanding Psychiatric Disorders

Neurotransmitters play a crucial role in regulating mood, attention, appetite, and cognition. Psychiatric disorders are often associated with imbalances in neurotransmitter levels. Three key neurotransmitters—serotonin, norepinephrine, and dopamine—impact various aspects of mental health. An imbalance in these neurotransmitters can lead to psychiatric disorders. (Association, 2013)

Serotonin, often referred to as the "feel-good" neurotransmitter, plays a crucial role in mood regulation. Low levels of serotonin are commonly associated with depression. Selective serotonin reuptake inhibitors (SSRIs), a class of antidepressant medications, work by increasing serotonin levels in the brain. Dopamine is involved in motivation, pleasure, and reward processing. Dysregulation of dopamine neurotransmission has been linked to depressive symptoms, particularly those related to anhedonia (loss of interest or pleasure). Norepinephrine is involved in the body's stress response and regulates mood. Drugs that increase norepinephrine levels, such as serotonin-norepinephrine reuptake inhibitors (SNRIs), are commonly used to treat depression. (Rush, 2006)

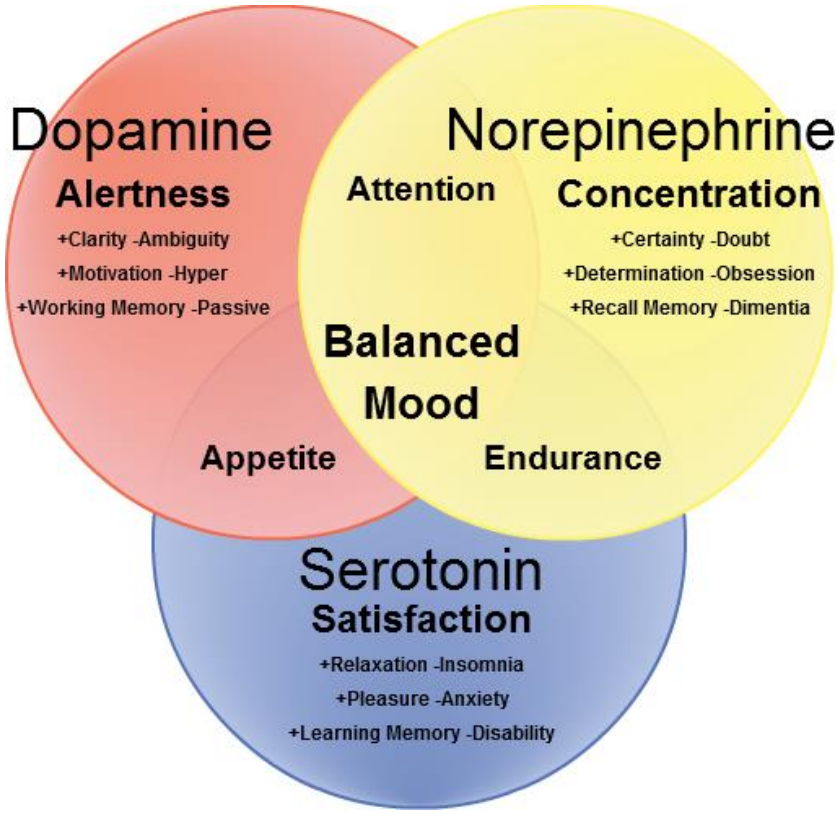

Figure 1: Venn diagram of neurotransmitters associated with depression.

2.3. Mitochondrial Cytochrome C Oxidase (CCO) and PBM:

Mitochondrial Electron Transport Chain (ETC) is a complex series of protein complexes and electron carriers essential for cellular energy production. In the context of psychiatric disorders, the inhibition of Cytochrome C Oxidase (CCO) by nitric oxide (NO) in damaged cells disrupts ATP production. Photo biomodulation (PBM) offers a novel

therapeutic approach by leveraging red to near-infrared (NIR) light to dissociate inhibitory NO from CCO, restoring cellular function. This is particularly relevant to brain disorders as neural tissues rich in mitochondrial CCO can benefit from PBM therapy. (Karu, 2010) (Chen, 2018) (Liebert, 2014) (Wang, 2017)

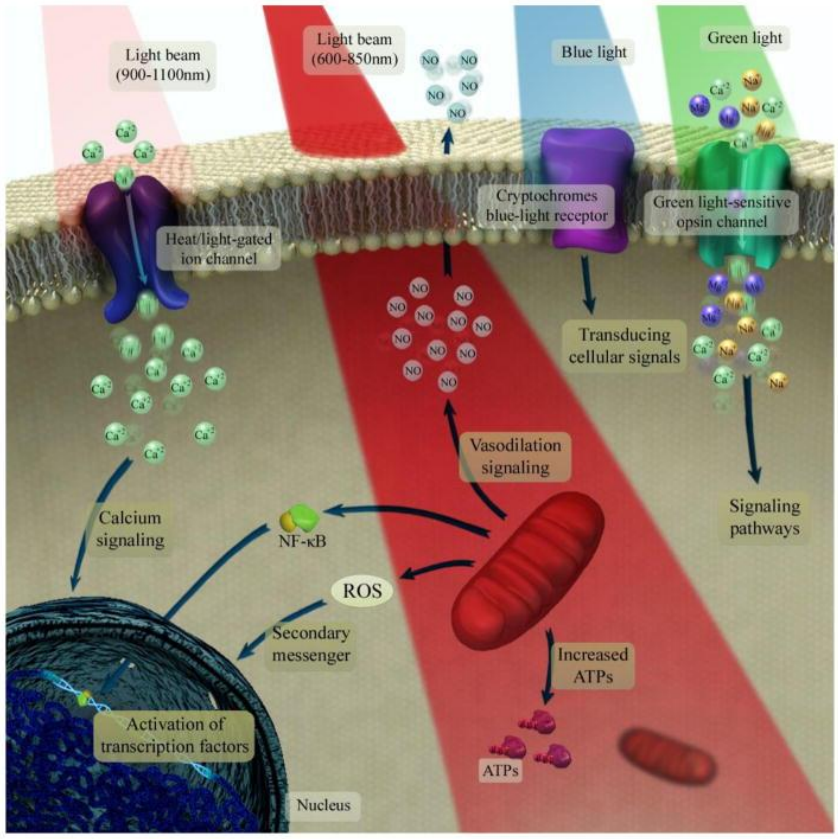

Figure 2: Cellular activity due to interaction of Red and Infrared light with the cell.

2.4. Benefits of Photo biomodulation:

PBM exhibits multifaceted benefits, including vasodilation, increased blood flow, modulation of immune cells towards anti-inflammatory responses, stimulation of cell proliferation, tissue regeneration, and neuroprotection. By influencing neurotransmitter levels and enhancing cellular function, PBM presents a promising avenue for addressing psychiatric disorders through non-invasive means. (Cacciatore, 2014) (Karu, 2010)

2.5. Parameters for PBM:

PBM treatment requires light source with some wavelength of light. To achieve this different source can be used which can be LASER based or LED based. It was found that compared to red light (wavelength 633nm), NIR light (830nm) more appreciably penetrated the skull and soft tissue. In case of LED NIR emitters with lower energy (50 and 200mW) source experiments by Henderson and Morris (2015) has shown penetration of the light into the skull for about 3cm tissue with attenuation of around 95%. Different high-power source of around 5-10W has shown significant penetration into the tissues. As the wavelength increases the penetration also increases. Experiments were done to test the effect of pulsation of the light source. It was observed that the pulsation at (10 Hz) led to higher energy delivery. To capitalize on the brain's capacity for photon scattering, Yue et al. (2015) suggested deploying a multiunit emitter array spaced equally over the scalp. They tested this theory by running Monte Carlo simulations on a model of the human head and showing that the multiunit emitter increased photon flux and improved uniformity of distribution. (Cacciatore, 2014) (Naeser, 2014)

2.6. Summarising The Literature Findings

TPBM is a simple, non-invasive option for managing and lessening depression. We can utilize PBM with NIR LEDs that have a wavelength of 830 nm. It is also possible to modify several factors such as timing, intensity, and pulsation to control the precise amount of power applied to the tissue. Additionally, there were hints about how to distribute the light source such that the brain receives greater photon influx. All these factors will be considered and included into the gadget design during the design process. (Demidova-Rice, 2010) (Cassano, Henderson and Morris experiments on penetration of NIR light into the skull, 2016)

2.7. Validation of the device.

Using animal model Zebra fish (Danio rerio) we can induce depression by treating the fishes with CUS (chronic unpredictable stress) protocol. A 15 days CUS protocol can significantly induced depression in zebra fish. Two distinct stressors were applied to zebrafish daily for 15 days as part of a CUS paradigm. Behavioural studies were conducted to evaluate anxiety and associated mood disorder characteristics using the novel tank test. This zebrafish

model also showed decreased neurogenesis, which is another indicator of anxiety and associated problems in fishes. (Porsolt, 1978) (Luchiari, 2016)

Once the fishes are depressed, we will be treating them using PBM with our developed device and specialised applicator.

Chapter 3: Materials and Methods

3.1. PBM Device Fabrication:

3.1.1 Head Unit Design and Fabrication:

The head unit of the PBM therapy device serves as the primary interface for delivering precise and targeted near infrared (NIR) light therapy to the frontal region of the brain. Designed with both functionality and user comfort in mind, the head unit features a concave-shaped panel accommodating 24 IR LEDs strategically placed to optimize light delivery. This design enhances the effectiveness of Photo Biomodulation (PBM) therapy by focusing on specific brain regions associated with affective disorders. The unit is constructed using 3D printing technology, ensuring a lightweight and adjustable structure that can be comfortably worn on the head. Velcro straps add an extra layer of adjustability, allowing the user to secure the device with ease. The careful engineering of the head unit reflects a commitment to precision in achieving therapeutic outcomes while prioritizing user convenience.

3.1.1. A. Materials:

- 3D rendering software (Sketch Up)
- IR LEDs (830 nm wavelength)
- Lightweight and durable material for the head unit (PLA plastic)
- Velcro straps
- Wiring and connectors

3.1.1. B. Methods:

Designing the Head Unit:

- Utilize 3D rendering software to design a concave-shaped panel for mounting 20 IR LEDs.
- Design a band for holding the panel securely on the head.
- Integrate Velcro straps for easy adjustment.

  IR LED Configuration:
- Connect 24 IR LEDs in total (3 LEDs in each series).

- Connect 8 series in parallel for increased power output.

Fabrication:

- Use the designed 3D model to fabricate the head unit using lightweight and durable materials PLA
- Install wiring and connectors to connect the LEDs in the desired configuration.

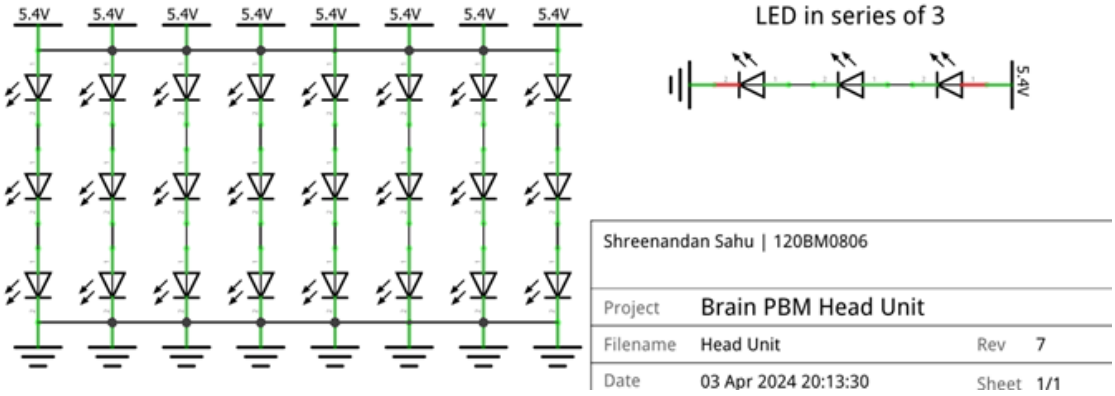

Figure 3: Circuit Schematics explaining the connections of the LEDs for the head unit.

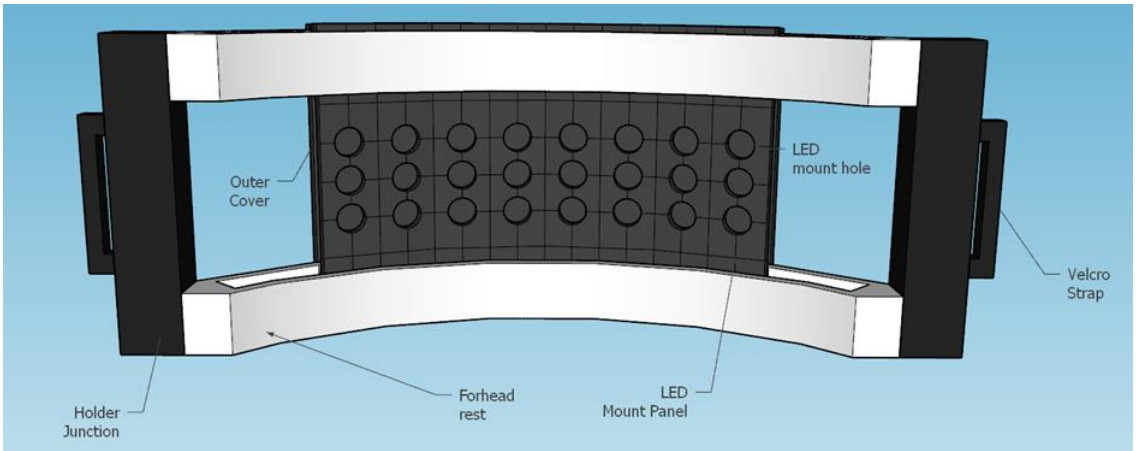

Figure 4: 3D rendered design of the Head Unit with parts levelled (Front View)

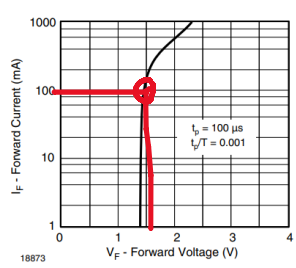

Fig. 4 - Forward Current vs. Forward Voltage

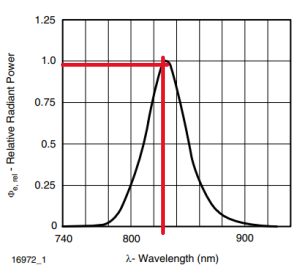

Fig. 7 - Relative Radiant Power vs. Wavelength

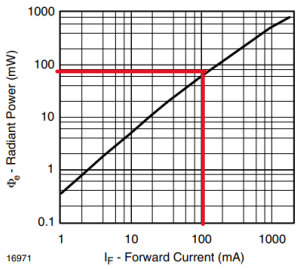

Fig. 6 - Radiant Power vs. Forward Current

Figure 5: Plot showing the variation in the parameters as the voltage and current varies.

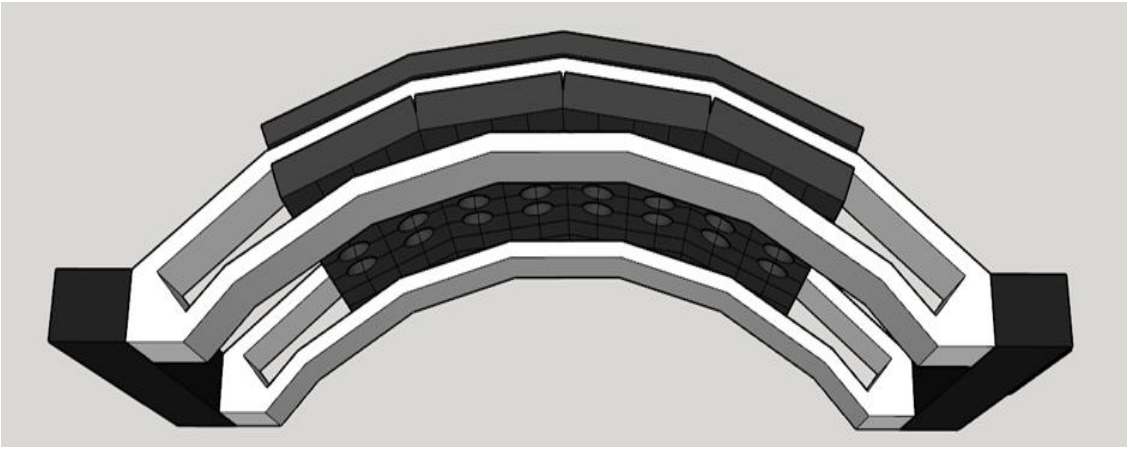

Figure 6: 3D rendered design of the Head Unit (Top View)

BASIC CHARACTERISTICS (T_{amb} = 25 °C, unless otherwise specified)

PARAMETER	TEST CONDITION	SYMBOL	MIN.	TYP.	MAX.	UNIT
Forward voltage	I_F = 100 mA, t_p = 20 ms	V_F		1.5	1.8	V
	I_F = 1 A, t_p = 100 µs	V_F		2.3		V
Temperature coefficient of V_F	I_F = 1 mA	TK_{VF}		- 1.8		mV/K
Reverse current	V_R = 5 V	I_R			10	µA
Junction capacitance	V_R = 0 V, f = 1 MHz, E = 0	C_j		125		pF
Radiant intensity	I_F = 100 mA, t_p = 20 ms	I_e	45	70	135	mW/sr
	I_F = 1 A, t_p = 100 µs	I_e		700		mW/sr
Radiant power	I_F = 100 mA, t_p = 20 ms	ϕ_e		50		mW
Temperature coefficient of ϕ_e	I_F = 100 mA	$TK\phi_e$		- 0.35		%/K
Angle of half intensity		φ		± 22		deg
Peak wavelength	I_F = 100 mA	λ_p		830		nm
Spectral bandwidth	I_F = 100 mA	$\Delta\lambda$		40		nm
Temperature coefficient of λ_p	I_F = 100 mA	$TK\lambda_p$		0.25		nm/K
Rise time	I_F = 100 mA	t_r		20		ns
Fall time	I_F = 100 mA	t_f		13		ns
Cut-off frequency	I_{DC} = 70 mA, I_{AC} = 30 mA pp	f_c		18		MHz
Virtual source diameter		d		3.7		mm

Figure 7 Data sheet of the NIR LED used in the development.

3.1.1. C. Parameter Calculations:

- Power Supply Voltage: 6.0 V (2 * 3.75 V-1.4V)
- LED Configuration:
- Wavelength: 830 nm
- Forward Voltage (Vf): 1.5 V-1.8v
- Forward Current (If): 100 mA
- Total Emitted Power per LED (P): (50 mw)

Series Connection:

- Number of LEDs in Series:
- Total Voltage Drop for the Series (Vs): 5 * Vf = 3 * 1.8 V = 5.4 V
- Total Power for the Series (Ps): 3 * P = 3 * 0.05 W = 0.15 W (150 mW)

Parallel Connection:

- Number of Series Sets in Parallel: 8
- Total Current Draw (I total): 8 * If = 4 * 100 mA = 800 mA (0.8 A)
- Total Energy per Second (Total): 8 * Ps = 8* 0.15 W = 1.2W (1200 mW)

The head device will be powered by a 6.0 V supply consisting of 3 LEDs in series, repeated 8 times in parallel 24 in total.

This configuration will draw a total current of 800 mA and produce 1200mJ of energy per second.

These calculations assume ideal conditions, and real-world performance may vary based on factors such as LED tolerances and overall circuit efficiency.

3.1.2 Pocket Unit Design and Fabrication:

The head unit of the PBM therapy device serves as the primary interface for delivering precise and targeted infrared (IR) light therapy to the frontal region of the brain. Designed with both functionality and user comfort in mind, the head unit features a concave-shaped panel accommodating 20 IR LEDs strategically placed to optimize light delivery. This design enhances the effectiveness of Photo Biomodulation (PBM) therapy by focusing on specific brain regions associated with affective disorders. The unit is constructed using 3D printing technology (creality ender S5 pro), ensuring a lightweight and adjustable structure that can be comfortably worn on the head. Velcro straps add an extra layer of adjustability, allowing the user to secure the device with ease. The careful engineering of the head unit reflects a commitment to precision in achieving therapeutic outcomes while prioritizing user convenience.

3.1.2. A. Materials:

- Lithium-ion rechargeable batteries (3.7 V, 2500mAh)
- 7808 voltage regulators ICs
- Indicator components (Red LED, Blue LED, Buzzer)
- Bluetooth communication module (HC-05)
- 3D rendering software SketchUp
- Wiring and connectors

- Push button Switch

3.1.2. B. Methods:

- Power Supply Design:
  - Connect two lithium-ion batteries in series to provide a total voltage of 7.5 V.
  - Use a 7809-voltage regulator for stepping down and regulating voltage at 9 V.
- Indicator Circuit Design:
  - Integrate red and blue LEDs for power and activity indication.
  - Include a buzzer for alerting the user to specific events.
- Bluetooth Communication:
  - Connect an HC-05 Bluetooth module for wireless communication.
  - Establish communication between the Bluetooth module and an Arduino Nano microcontroller.

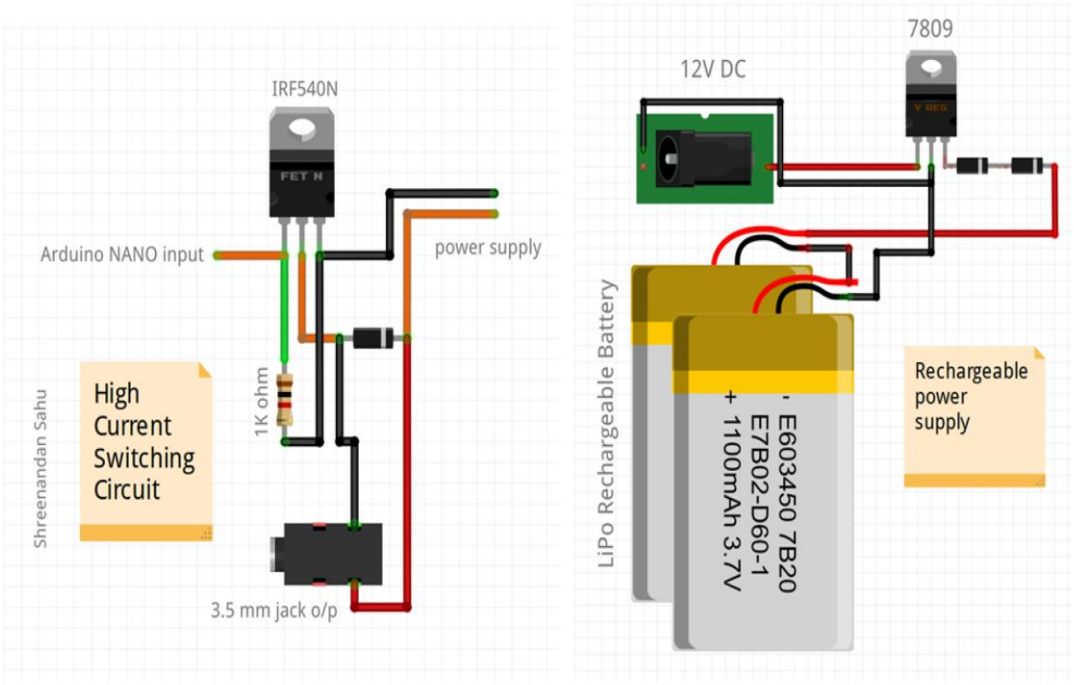

Figure 8: circuit diagram of the switching circuit and battery connection with circuit

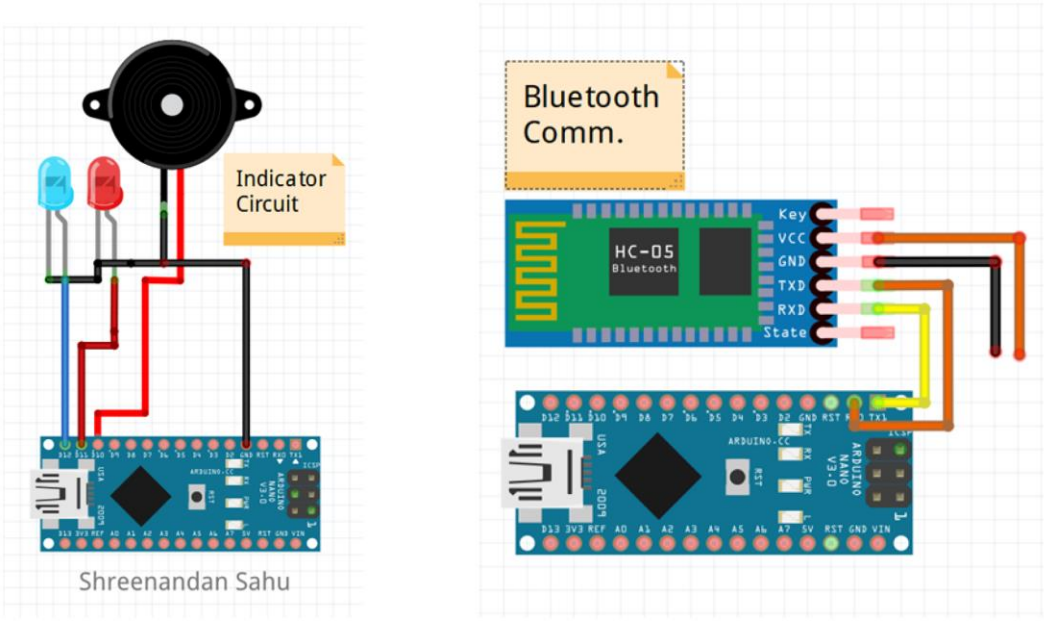

Figure 9: circuit diagram of the indicator circuit and Bluetooth connection for communication with app.

Figure 10: Orthogonal view of rendered Pocket Unit (initial model)

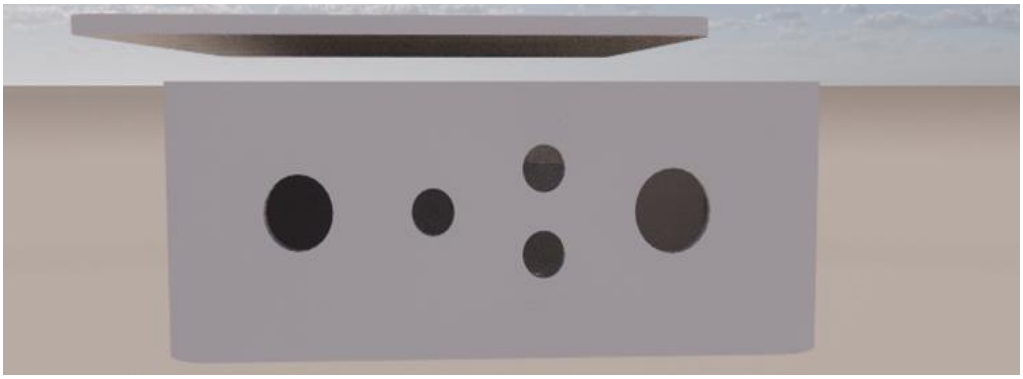

Figure 11: Front View of rendered Pocket Unit (initial model)

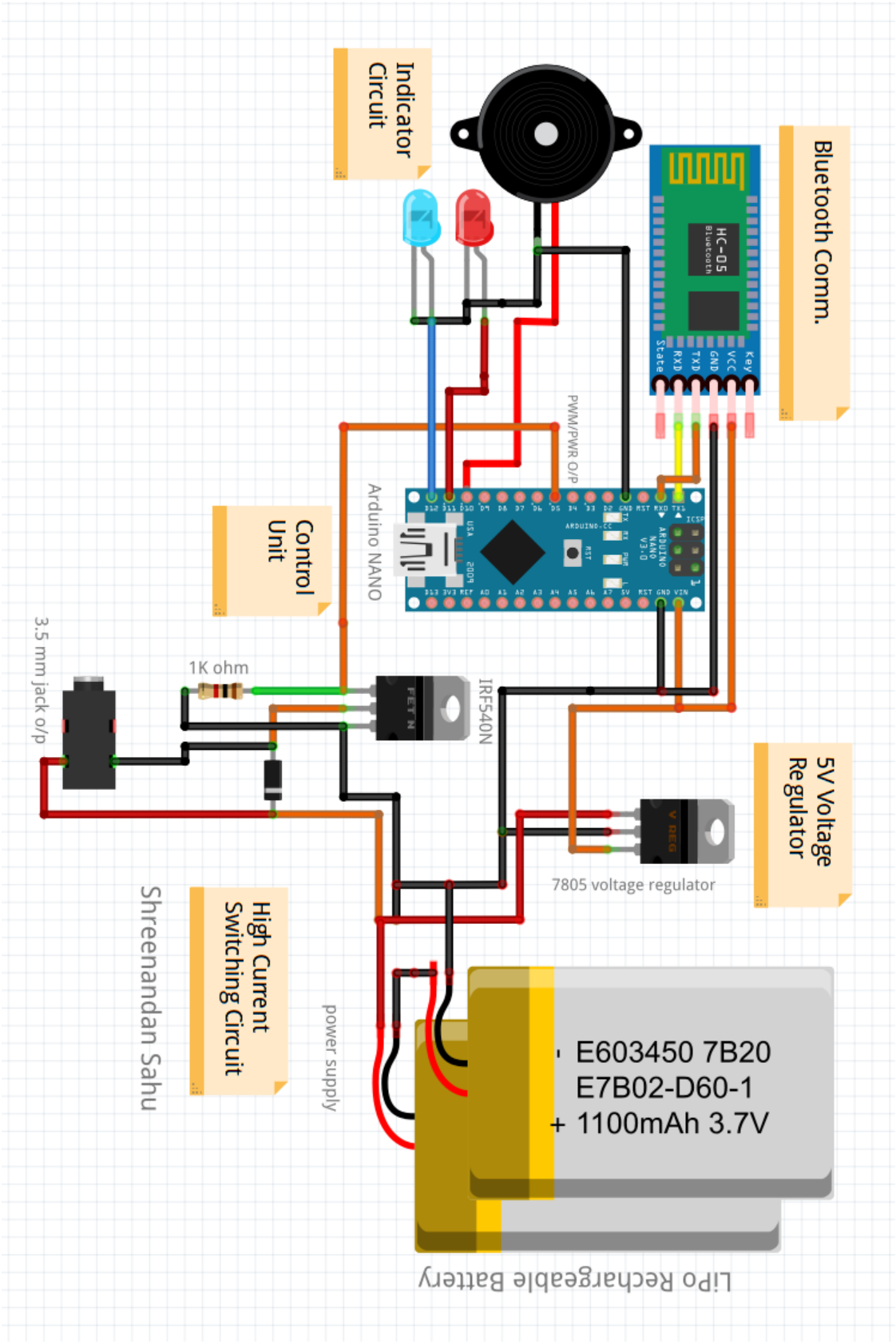

Figure 12: Final circuit diagram of the initial model with hardwiring and Arduino Nano as the main microcontroller.

3.1.3 Pocket Unit Redesigning forATMEGA328P.

For efficient performance we redesigned the pocket unit. This time we have used ATMEGA28P as the controlling microcontroller and designed a custom PCB for the entire circuitry to enhance the performance and efficiency. The PCB was designed using Fritzing software and was fabricated by PCB Power pvt. ltd. The PCB was miniaturized and was made compact by eliminating 3rd part hardware. The new size of circuitry was enclosed in a small area of 5cm x 6cm. We also used a separate charging module for handling the charging of the rechargeable batteries. The model we used is (2S 10A 18650 7.4V-8.4V Lithium Battery Protection Board). We have used 2 LiPo 18650, 3000mAh battery for the operation of the entire circuit. These rechargeable batteries are light weight and are compact giving the device light weight and compactness. We have included a charging port and charging indicator along with Zennor diode to stop reverse flow of current. The output of the device is given using a 3.5mm audio jack. A 3.5mm audio cable can be used to connect the two units. Overall expected voltage output of the device is about 6V which is enough to run 3 NIR LED of 830nm connected in series. To make different applicators we can combine different numbers of these 3 series connections in parallel to enhance the power output of the LED light. These batteries can supply enough current to keep the device running for about an hour.

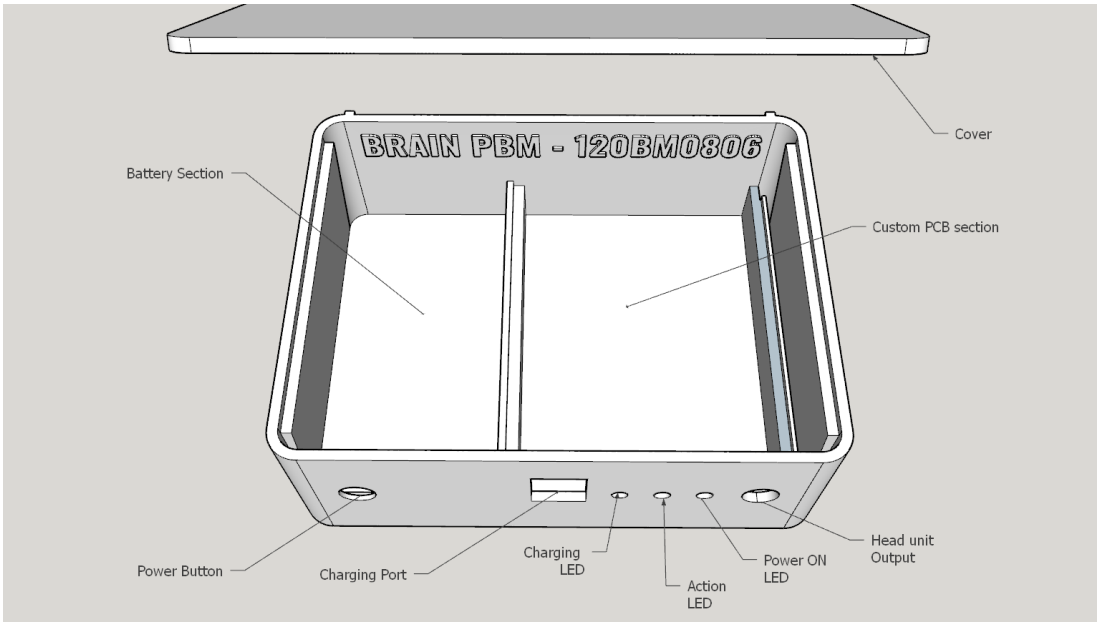

Figure 13: levelled Front view of rendered Pocket Unit (final model)

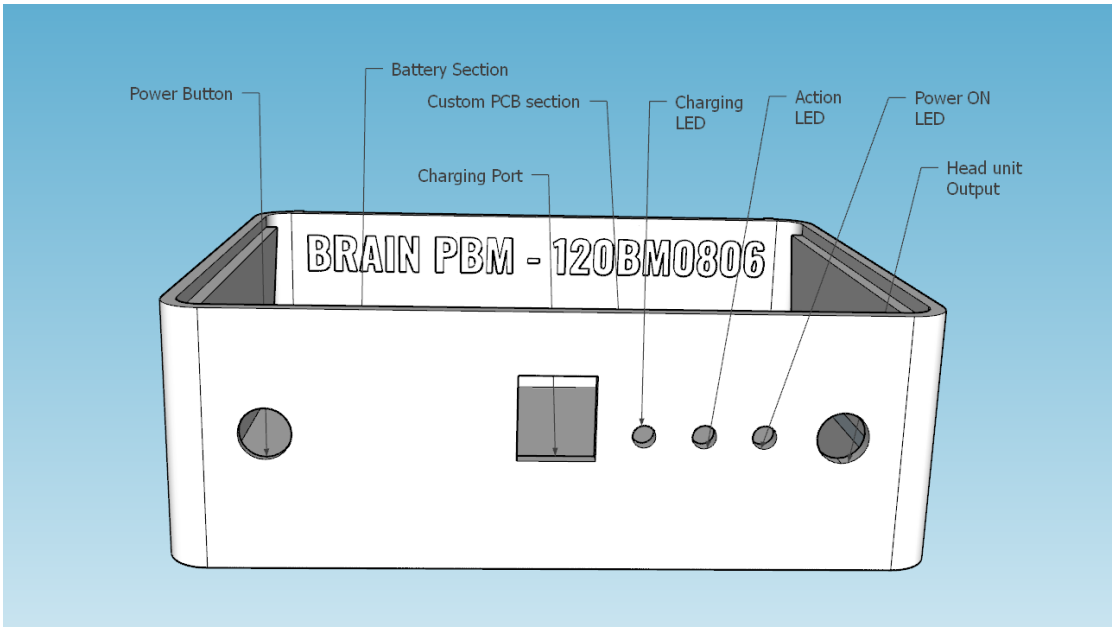

Figure 14 : levelled Front view of rendered Pocket Unit (final model)

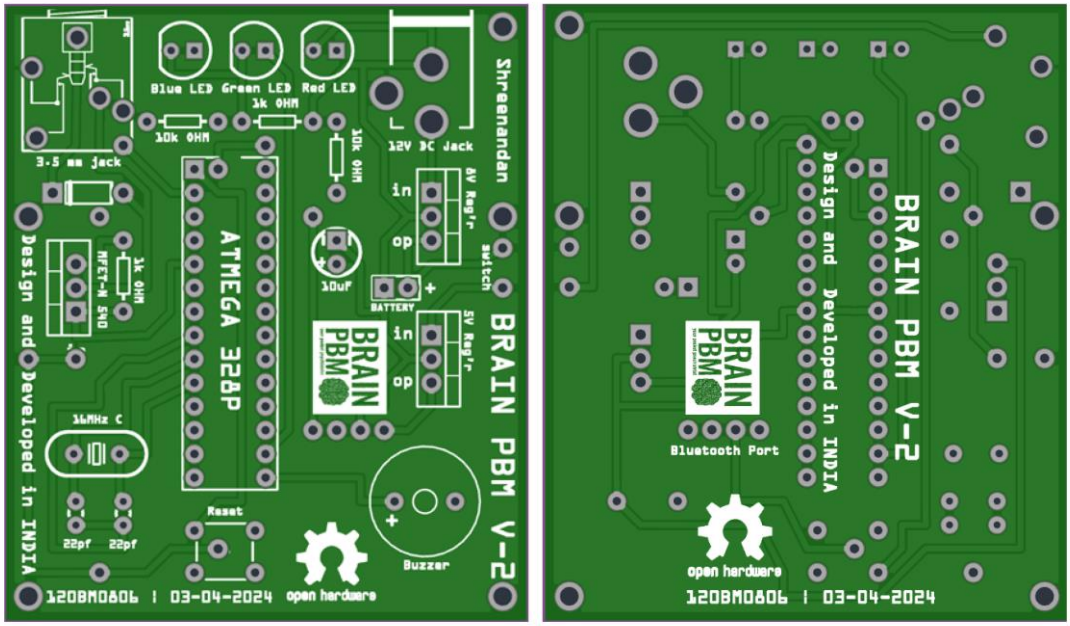

Figure 15: rendered image of the final custom PCB designed for the pocket unit.

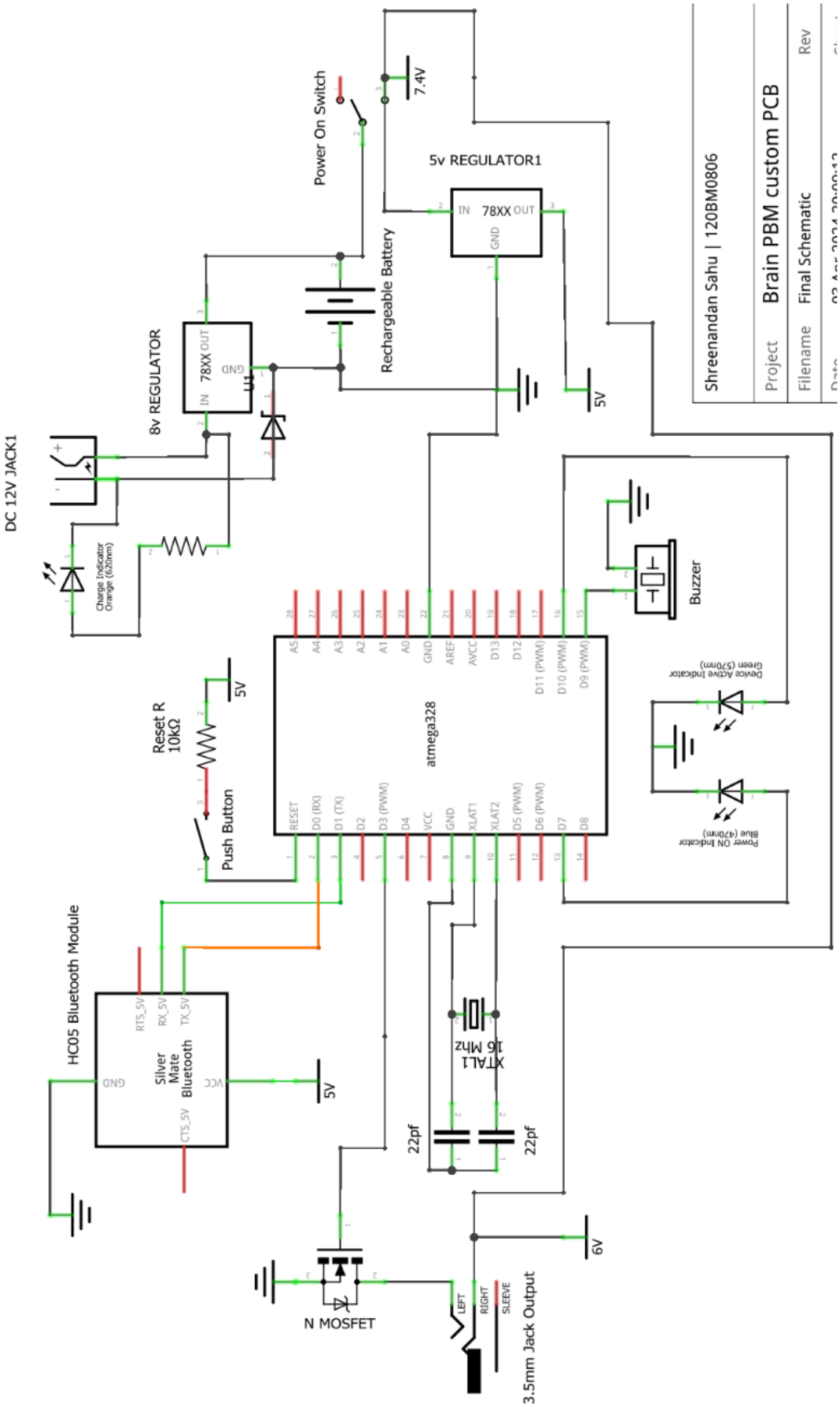

Figure 16: Final circuit diagram of the custom PCB for the control unit housing all the components.

3.1.4 App Design and Development

The primary goal of developing a mobile app for the Brain device is to provide users with a user-friendly interface to control and monitor the photobiomodulation therapy. The app enables seamless communication between the user's mobile device and the BrainPBM device via Bluetooth.

The app is developed using the MIT App Inventor, an open-source, web-based platform for building Android applications. MIT App Inventor is chosen for its simplicity and visual programming environment, making it suitable for rapid prototyping and development.

3.1.4. A. Key Features:

A. *Operation Modes:*

- The app includes different operation modes such as Test Run, Full Run, Open Parameter, Fish run and an instructional How-to-Use page.
- Test Run mode allows users to check the functionality of the device.
- Full Run mode involves score-based treatment, where users input their BDI score, and the app provides options for different therapy modes.
- Open Parameter mode allows users to modify the wattage of the head unit by controlling the pulsation, intensity, and timing of the LEDs.
- Fish mode allows us to the pilot study on the zebra fish model.

B. *Bluetooth Connectivity:*

- The app facilitates Bluetooth communication between the mobile device and BrainPBM device.
- It lists available Bluetooth devices, allowing users to connect to the BrainPBM device seamlessly.

C. *User Interface*

- The app's main screen presents operation modes, and users can navigate to different screens based on their requirements.
- Intuitive buttons and controls ensure a user-friendly experience.

Test Run Screen:

- In Test Run mode, users can choose the BrainPBM device and execute a test, such as pulsing at specific frequencies for a predefined duration.

Full Run Screen:

- In Full Run mode, users input their BDI score, and the app provides options for different therapy modes, allowing users to choose between pulsatile and continuous modes.

Open Parameter Run Screen:

- This screen has connected to device button to connect to the device and 3 more placeholders for frequency, time and intensity of the Head unit.

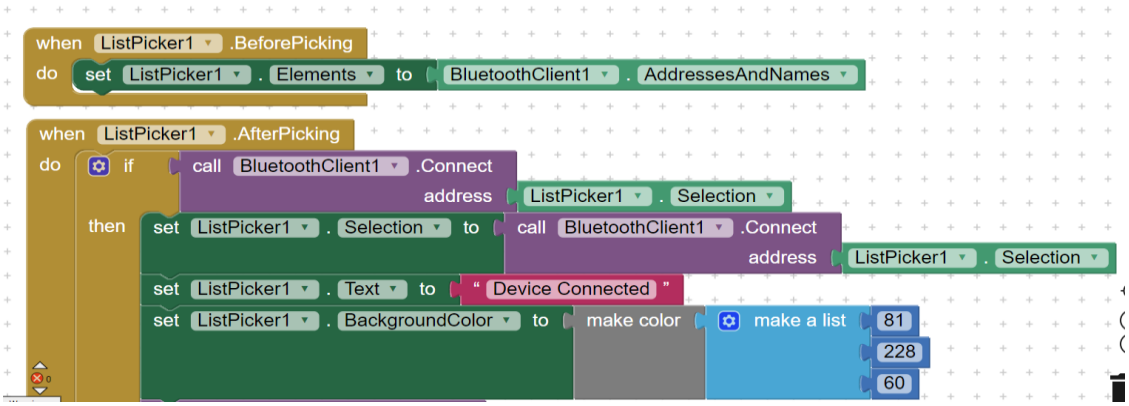

Figure 17: Blocks of logic used for app development 1

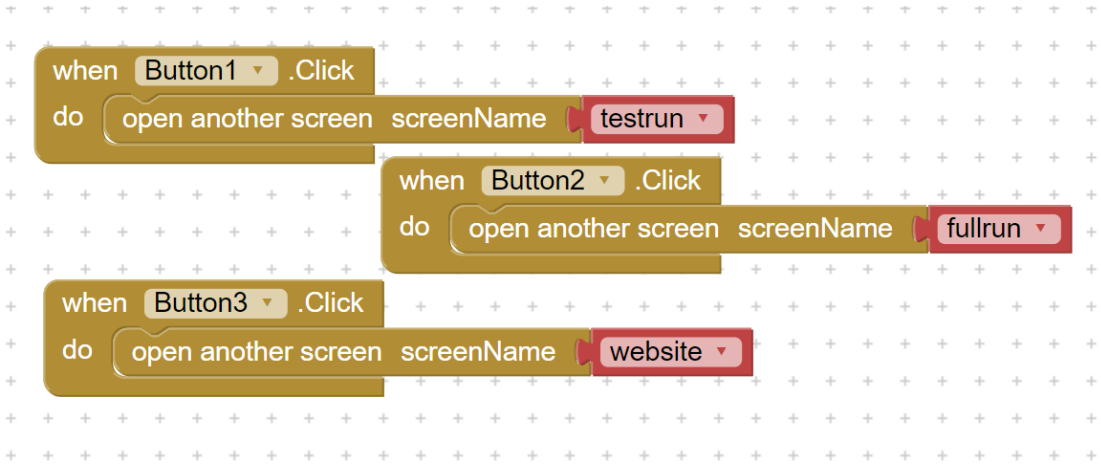

Figure 18: Blocks of logic used for app development 2

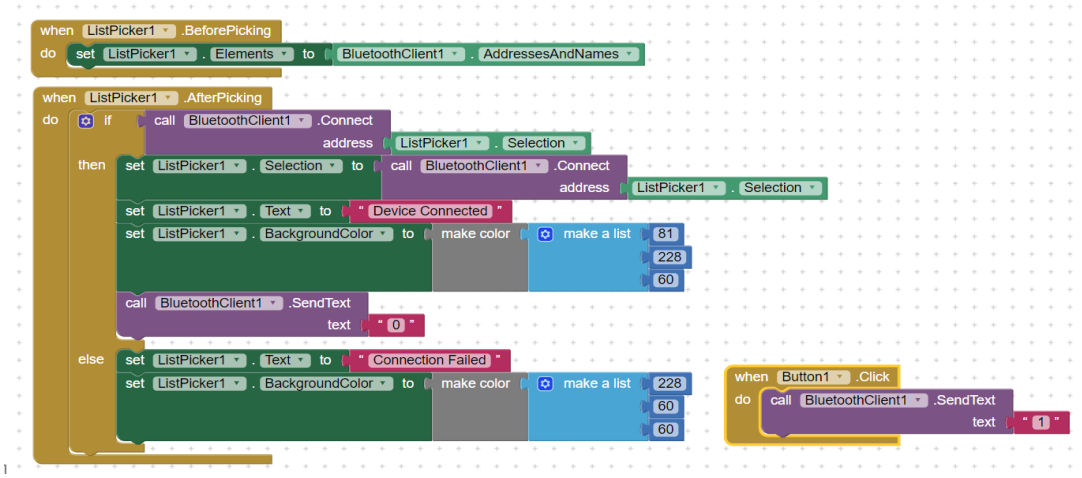

Figure 19: Blocks of logic used for app development 3

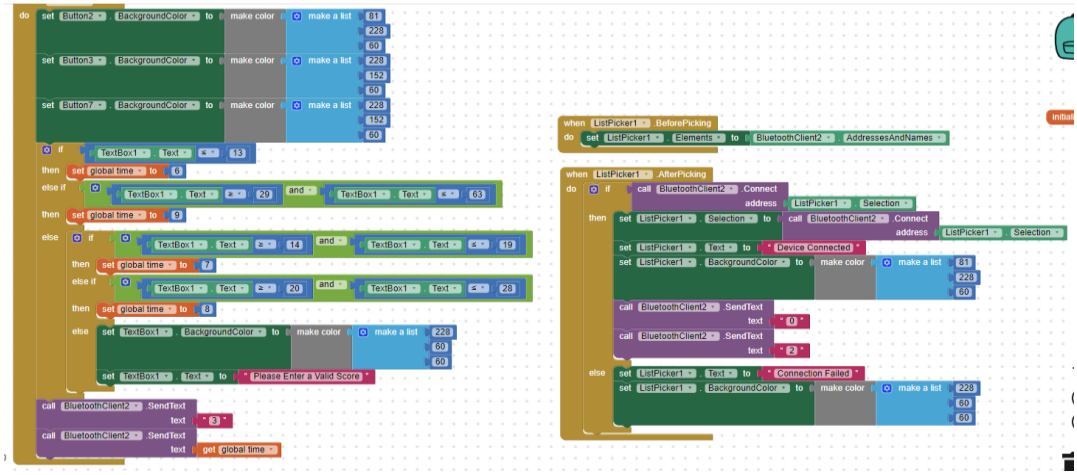

Figure 20: Blocks of logic used for app development 4

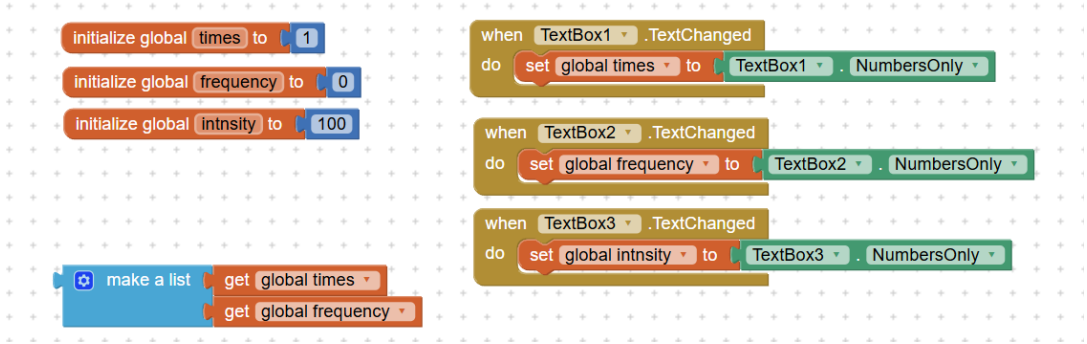

Figure 21: Blocks of logic used for app development 5

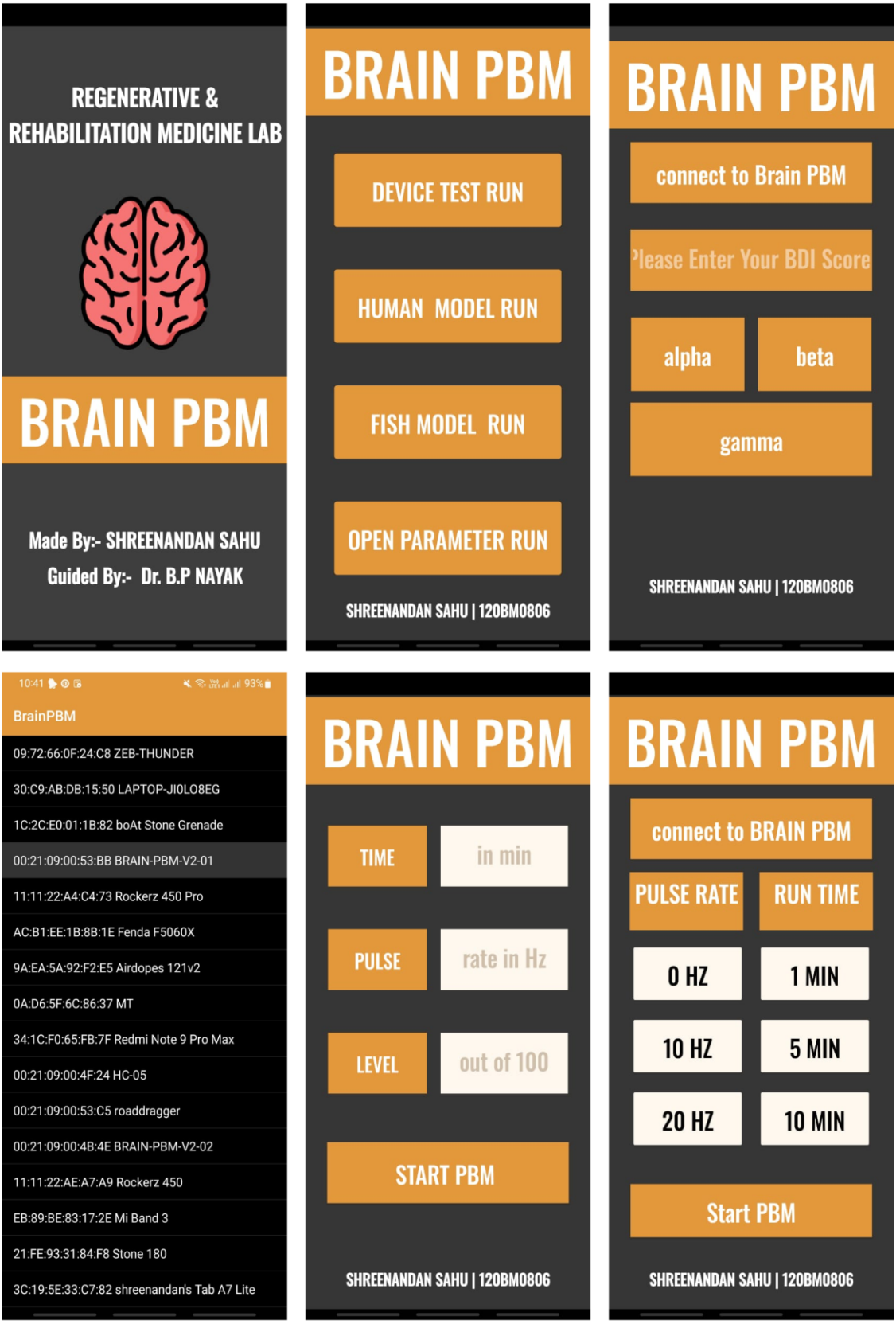

Figure 22: UI screens of all the scren present in the mobile app.

3.2. Experiment Design for Device Validation

For validation of the device, we will be using animal model i.e., Zebra fish model (Danio rerio). Initially we will be treating them with chronic unpredictable stress (CUS) for 15 days using different stressors and then we will be treating them using PBM therapy using our device for the next 15 days. The CUS protocol is described below.

3.2.1 CUS PROTOCOL

Table 1: Stressors applied to the fishes for inducing depression in them.

Sl. No	Protocol Name	Description
1	Lower water level (LWL)	The water level in the home tank is lowered until the dorsal body of the fish is exposed to outside water for 30 minutes.
2	Heat & Cold Stressor (HCS)	Fish are alternatively transferred from home tank to hot and cold-water tank maintained at 32°C and 23°C. Keeping them in each tank for around 5 minutes and repeating it for 10 times.
3	Net Chasing (NC)	Individually, each fish is chased with a net in a separate tank for 8 minutes.
4	Social Isolation (SI)	Individually, each fish is maintained in a 250 ml beaker with 150 ml water for 45 minutes.
5	Shaking (SH)	Individually, each fish was kept in an open falcon tube and was placed on vertexing machine for 10 minutes at 150 rpm.
6	Restraint (RE)	Fish were placed in a 50 ml water filled Falcon tube with openings on one side for breathing.
7	Turbulences (TUR)	Fish were kept in a tank and two bubblers were used to bubble the water for 10 minutes for individual fish.
8	Overnight illumination (ONI)	Fish were kept in a tank and were illuminated overnight using artificial lights.
9	Light flashes exposure (LFE)	Put the fish into a 70 ml dish (n = 30) with circulating water, first adapt to the dark conditions for 3 min, and then start flashing the lamp for 10 min (light flashes at 5 Hz).

10	Hyperosmotic shock (HOS)	put the fish into a 250 ml tank (n = 30) with 100 mM NaCl for 10 min, and then wash with circulating water for 3 min / time, three times in total.

Table 2: Timeline of application of stressors on the fishes

Days	Stress 1	Time	Stress 2	Time	Date
1	LWL	9:00AM	TUR	3:00PM	23rd March 2024
2	LFE	10:00AM	ONI	4:00PM	24th March 2024
3	NC	9:00AM	SH	3:00PM	25th March 2024
4	HOS	10:00AM	SI	4:00PM	26th March 2024
5	HCS	9:00AM	RE	3:00PM	27th March 2024
6	LWL	10:00AM	TUR	4:00PM	28th March 2024
7	LFE	9:00AM	ONI	3:00PM	29th March 2024
8	NC	10:00AM	SH	4:00PM	30th March 2024
9	HOS	9:00AM	SI	3:00PM	31st March 2024
10	HCS	10:00AM	RE	4:00PM	1st April 2024
11	LWL	9:00AM	TUR	3:00PM	2nd April 2024
12	LFE	10:00AM	ONI	4:00PM	3rd April 2024
13	NC	9:00AM	SH	3:00PM	4th April 2024
14	HOS	10:00AM	SI	4:00PM	5th April 2024
15	HCS	9:00AM	RE	3:00PM	6th April 2024

Table 3: Severity Classification of the applied Stressors

Mild Stressors	Moderate Stressors	Sever Stressors
Lower Water Level (LWL)	Heat & Cold Stressor (HCS)	Shaking (SH)
Overnight Illumination (ONI)	Net Chasing (NC)	Turbulences (TUR)
Social Isolation (SI)	Light Flashes Exposure (LFE)	Hyperosmotic Shock (HOS)
	Restraint (RE)	

3.2.2 PBM Treatment Protocol

After 15 days of CUS protocol, the depressed set of fishes will be treated with PBM using specially designed applicator. The applicator will be of 450mw, and we will be using the PBM device for treatment for about 20-30 minutes at full intensity and at 10Hz pulsation. The fishes will be treated twice daily with an interval of 6-8 hours for 15 days.

3.2.3 Behavioural Study of Zebra fish using ANY-MAZE

To analyse the behaviour of the fishes we will be using ANY-MAZE software which is industry standard and widely used for the motion tracking and behavioural study. We took 30 fishes and divide them into 2 groups. 10 as control group and 20 as CUS protocol group. As the CUS protocol is of 15 days we analysed the behaviours every 7th day starting from 0th day.

We used Novel Tank Test (NTT) for analysing the exploratory behaviour of the fishes. We used different parameters to analyse the fish behaviour which are mentioned in the table. The fish were taken out of home tank and then put into a novel tank which is new to them. 1 minute was given for the fishes to acclimatise into the new environment and then further 6 minutes (360 seconds) were used to record the behaviour of the fishes. Once recorded the ANY-MAZE software will automatically analyse the behaviour and according to the parameters set will give the results in the form of .txt file which for entire experiment has been collected into table and has been attached in the appendix section.

Table 4: Parameters for behavioral analysis in ANY-MAZE.

Parameters	Description of Parameters
Track Plot	Track plot shows the actual trajectory of the fish throughout the entire duration of recording.
Heat Map	Heat maps display the region which has been explored for the most amount of time. Higher the time spent intense the color of the region.
Average Speed	Describes the distance travelled by the fish per second. (m/s)
Freezing episode	The number of times the fish freezes in a particular region is called freezing episode. So, if a fish freezes for more than 45 sec it will be considered as a freezing episode.

Time spent in different zones	Explains the time spent by the fishes for the total of 360 seconds individual time spent by the fishes in upper zone and the lower zone will be considered.

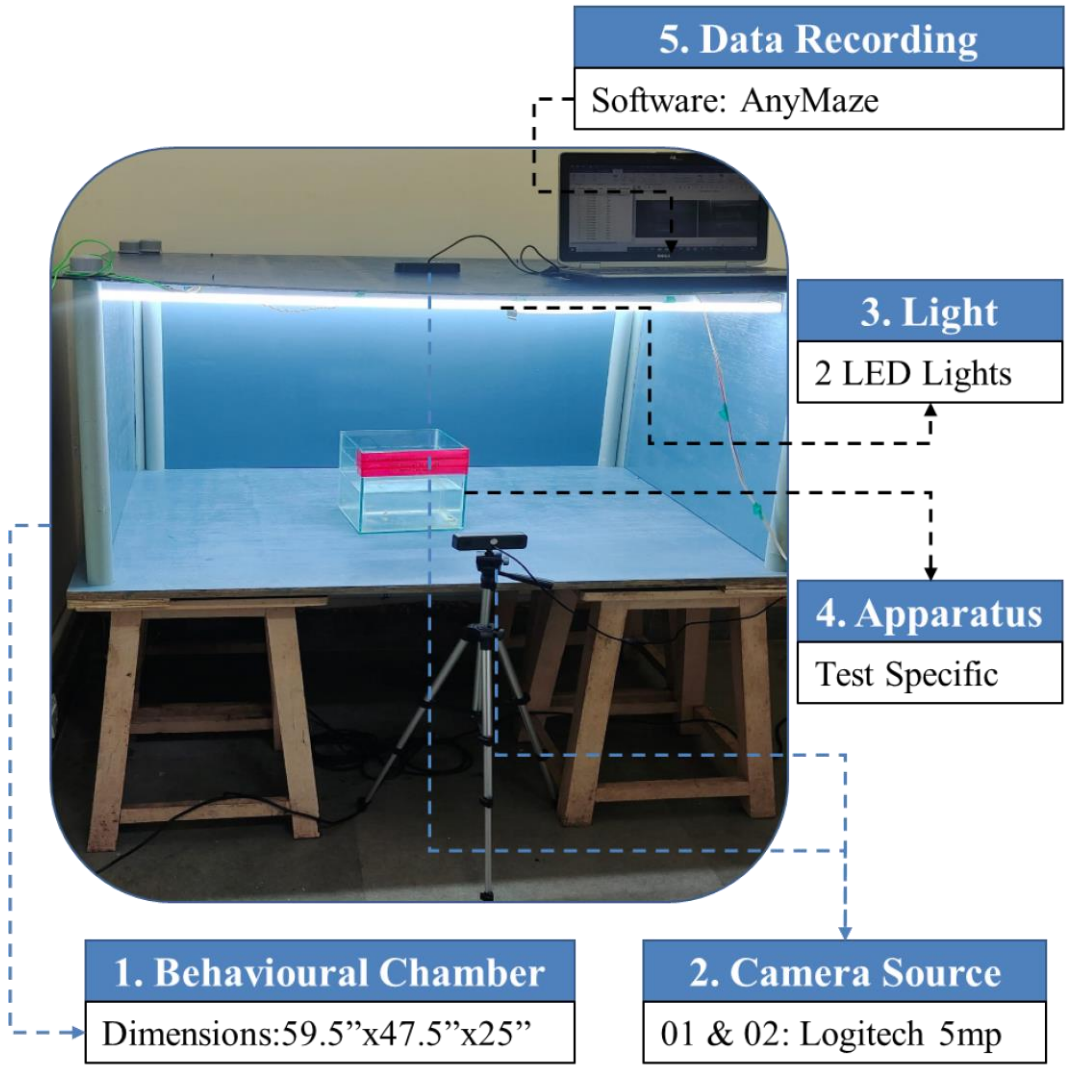

Figure 23: NTT setup for recording behavioral video of fishes.

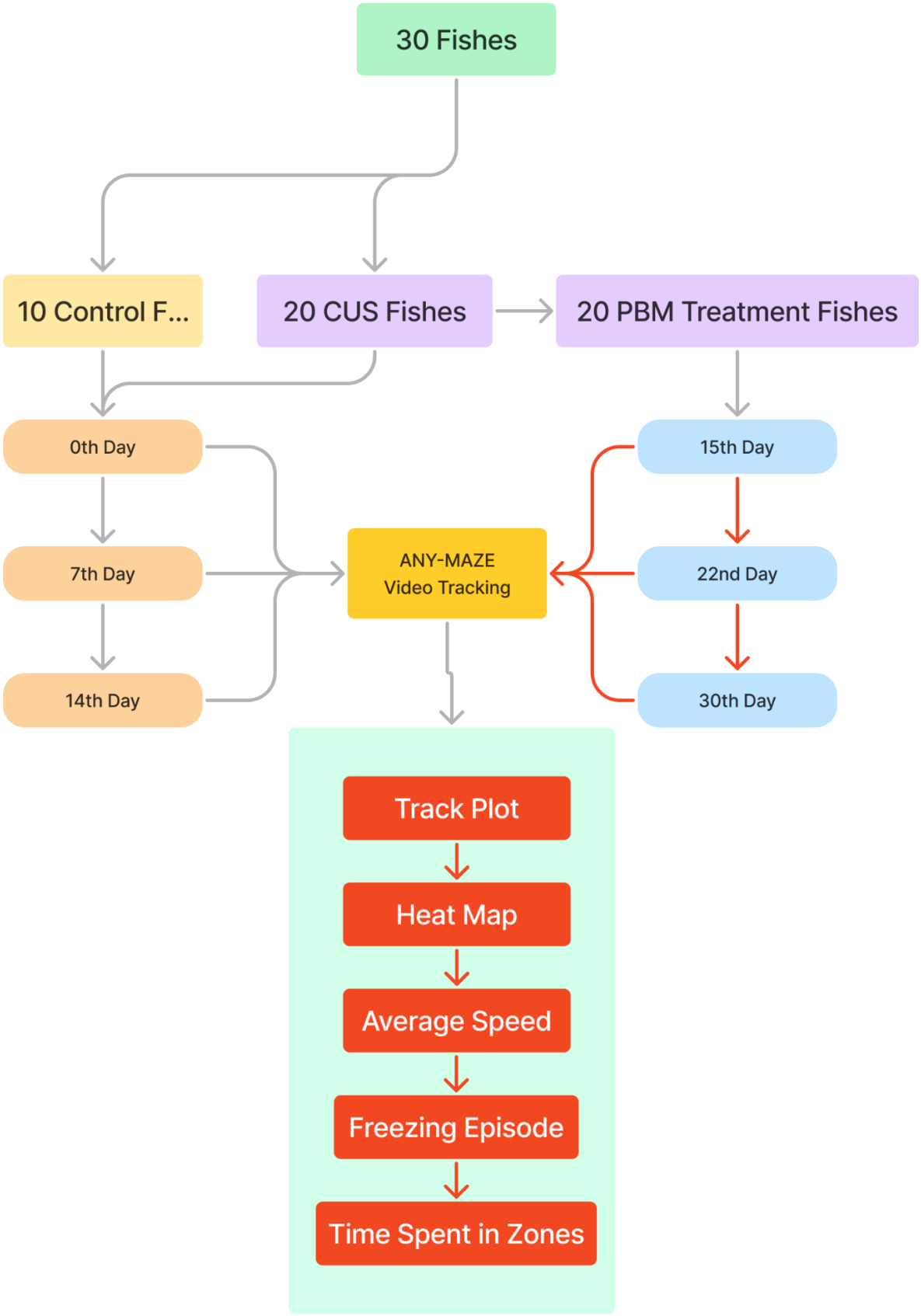

Figure 24: Flowchart of the validation experiment using zebra fish model.

Chapter 4: Results and Discussion

4.1. Head unit assembly:

The 3D designed head unit was printed and the LEDs were assembled into the LED panel. After mounting the LEDS they were trimmed and soldered, and the connections were established. After all the series and parallel connections, 2 wire were taken out and the 3.5mm jack was connected to the end. All the wires and the jack were cleared and were put behind the outer layer of the head unit. Support brackets were glued to the led panel and Velcro was attached to the support bracket from one side. This completed the head unit assembly.

Figure 25: completely mounted and assembled head unit (top), size of head unit compared to the palm (bottom)

4.2. Custom PCB and Pocket unit assembly:

The 3D designed head unit was printed and the LEDs were assembled into the LED panel. After mounting the LEDS they were trimmed and soldered, and the connections were established. After all the series and parallel connections, 2 wire were taken out and the 3.5mm jack was connected to the end. All the wires and the jack were cleared and were put behind the outer layer of the head unit. Support brackets were glued to the led panel and Velcro was attached to the support bracket from one side. This completed the head unit assembly.

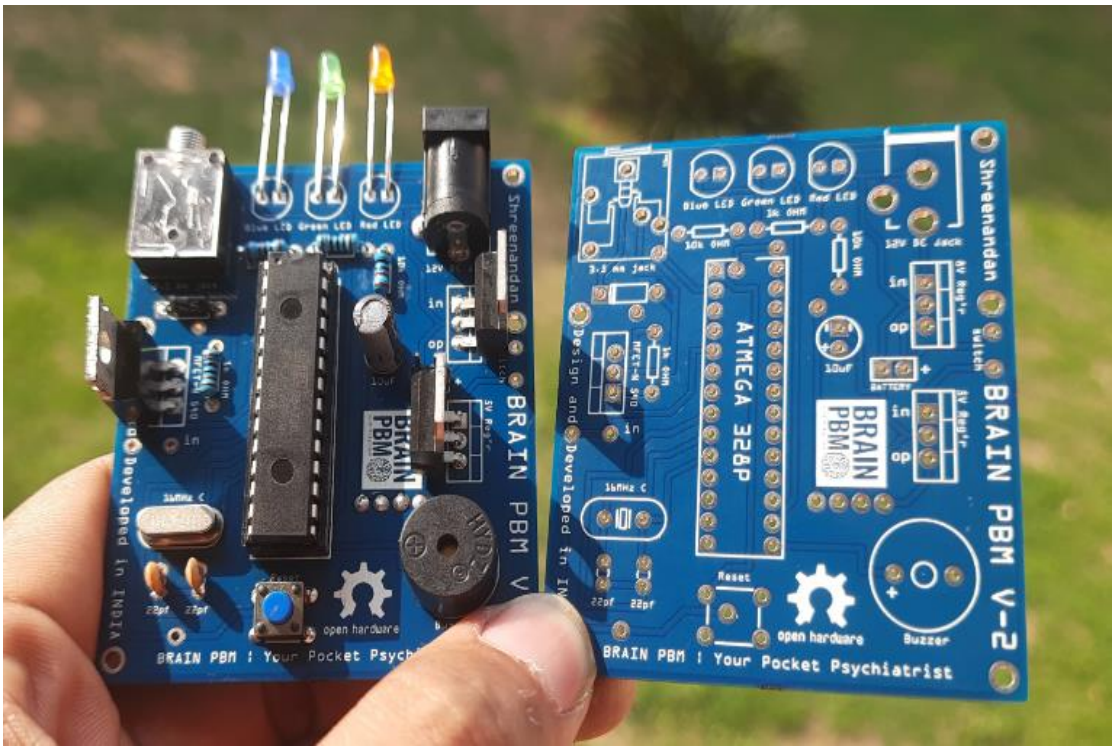

Figure 26: custom PCB, footprints of components (right), components after getting mounted on the PCB (left)

4.3. Head unit and Pocket unit integration with APP:

The two assembled units were connected to each other using a 3.5mm audio cable. The power switch was made ON and the device successfully started up.

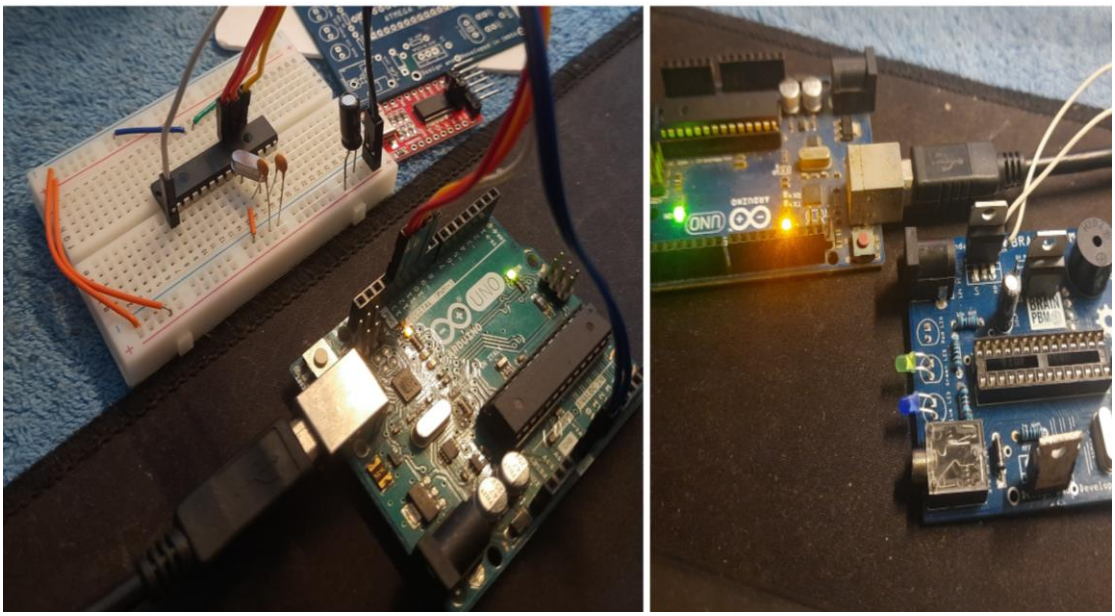

Figure 27: Burning the bootloader into the microcontroller (left) and programming the same (right)

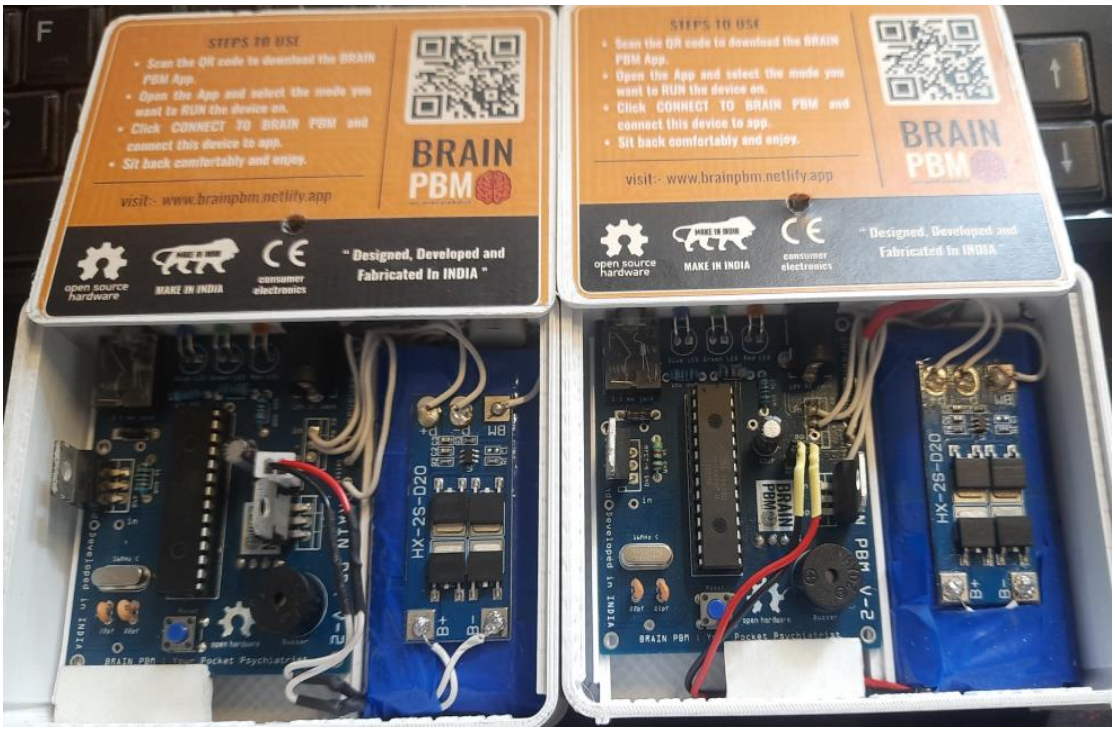

Figure 28: completely mounted and assembled pocket unit hosting custom PCB and the rechargeable batteries.

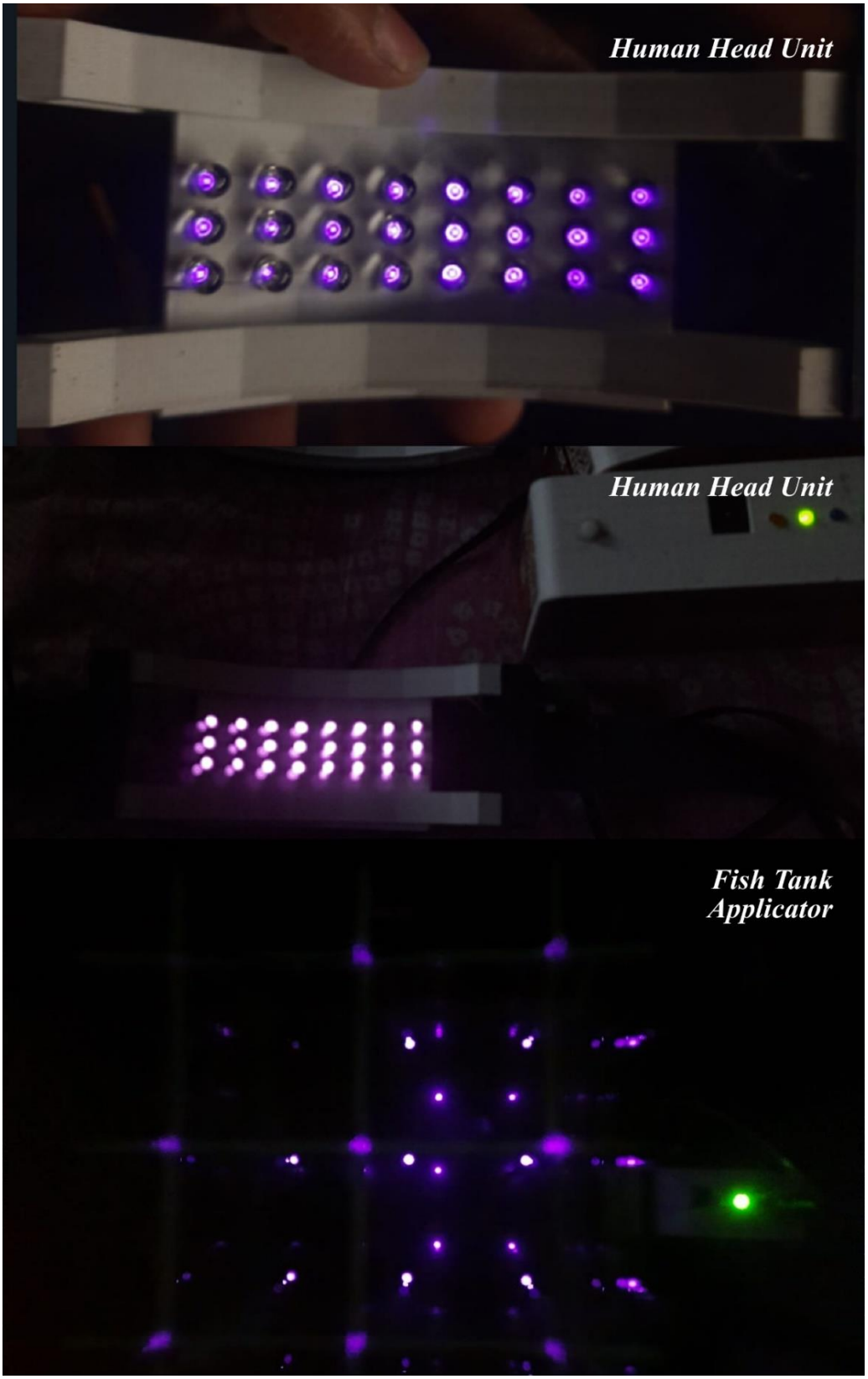

Figure 29: Fully functional device with different applicators. Human head applicator (top, middle) Fish tank applicator (bottom)

Figure 30: Completely fabricated and functional BRAIN PBM device with head unit (top) and pocket unit (bottom)

4.4. Pilot Study on Zebra Fish

10 fishes from the study group were taken for control study. They were put into NTT (Novel Tank Test) and their behavioral study was done using ANY-MAZE software. We obtained various parameters like average speed, number of freezing episodes and time spent in different zones. Results of these parameters are discussed below.

The left-out fishes were treated with Chronic Unpredictable Stress for 15 days using various stressors and were made depressed by the end of 15th day. The same set of fishes which were treated with CUS, were then treated with PBM for the next 15 days. We used the device and the specially designed fish tank applicator to apply PBM on fishes. The applicator had 9 bulbs giving a total output of 450mW. We treated the group of fishes with PBM therapy for 30 minutes twice daily for the next 15 days giving a total of 8.1kJ (8100 joules) of energy at every treatment session.

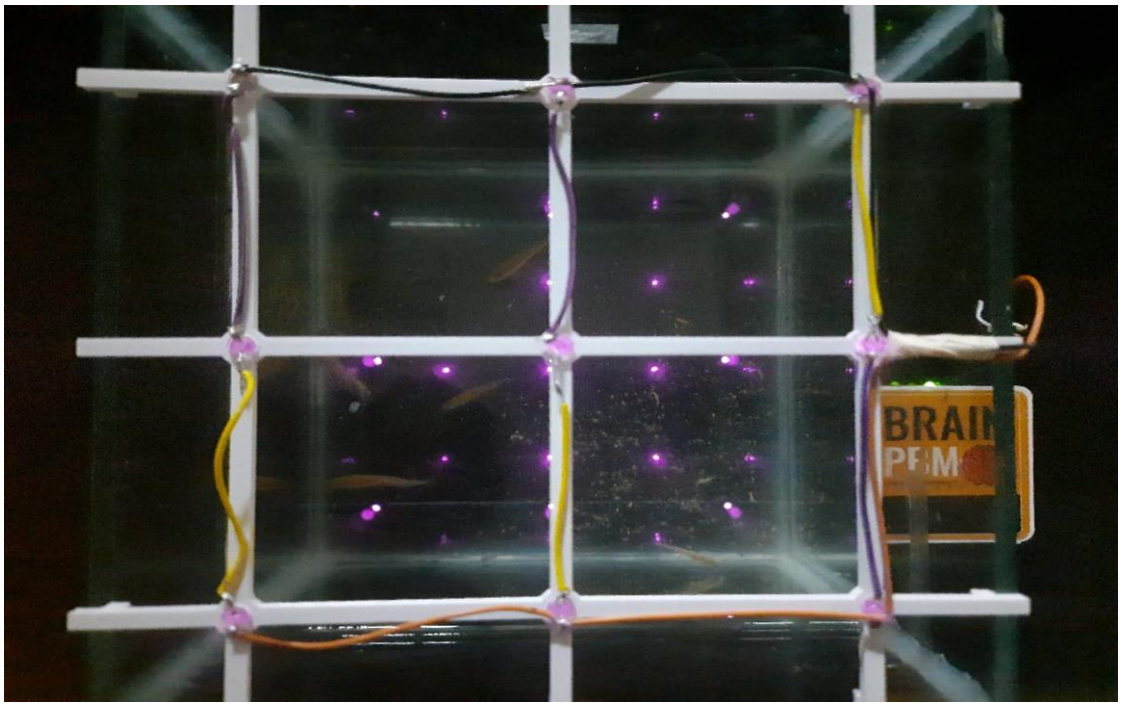

Figure 3131: Setup for PBM therapy for fish. Activated fish tank applicator (left) and pocket device (right)

NTT was also performed for these PBM treated fishes every 7th day starting from 0th day for the same 15 days. We analyzed their behavior using ANY-MAZE and found the following parameters like average speed, freezing episodes and time spent in different zones.

The following figure describes how the average speed of fish groups varies in comparison to the control group. It can be clearly seen that the average speed of the CUS treated group is decreasing from the control group which is around 0.0575m/s. As the CUS treatment proceeds the average speed decreases to 0.0368m/s. In contrast the PBM treated fishes have increment in the average speed from the initial average speed of 0.384m/s to 0.0589. This increment shows the positive effect of PBM treatment on the zebra fish model.

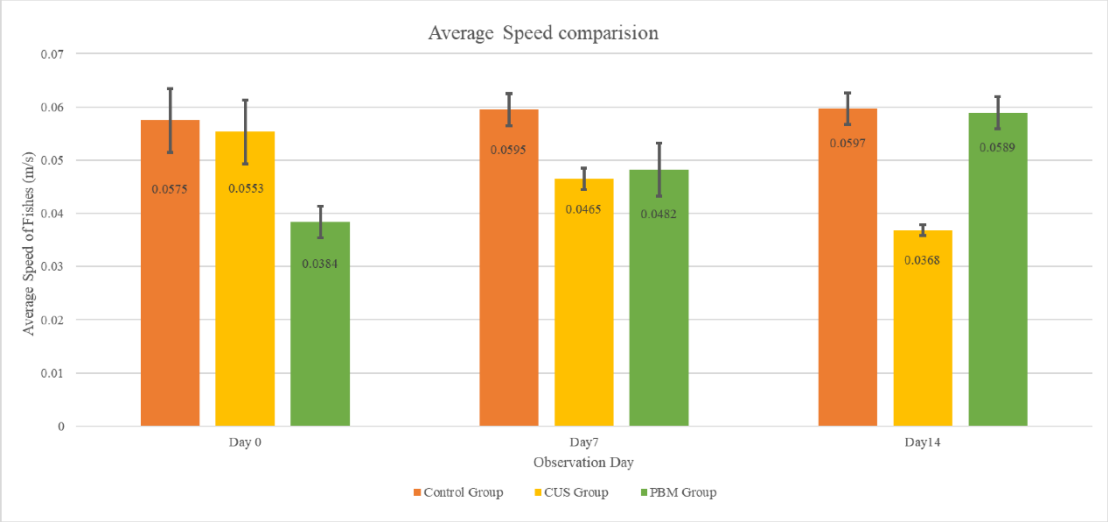

Figure 32: Plot for comparing average speeds of different groups on different days of observation.

The following figure describes how the average freezing episodes varies in the 3 different groups. For the control group there is no freezing as the fish are healthy and normal. On the other hand, the CUS treated fish group initially shows no freezing episodes but as the CUS continues by the 7th and 14th day the average freezing episodes increases to 2.9. On an average close to 3 episodes of freezing are present in tracking of 360 seconds which indicates depression in the CUS treated group. In the case of the PBM group on the 0th day of the treatment the average freezing episodes was initially 3.4 which over the period of treatment of 15 days decreased to around 0. This is a significant result and is proof that the PBM is helping in mitigating depression.

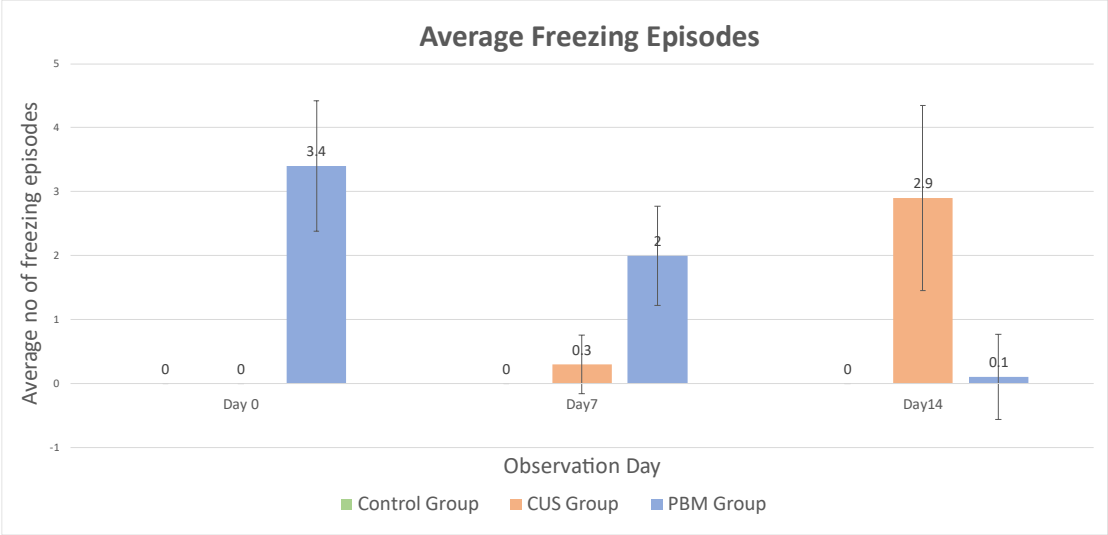

Figure 323: Plot for comparing average freezing episodes of different groups on different days of observation.

The following figure describes how the time spent in top and bottom layer varies. In normal conditions the time spent by the fish in both region is somewhat similar which is also evident in the figure below. But in the case of the depressed fishes the time spent in the bottom region increases in comparison to the top region. In the figure the CUS treated group is initially having similar time spent in the 2 regions, but as the protocol proceeds the time spent in the bottom layer increases.

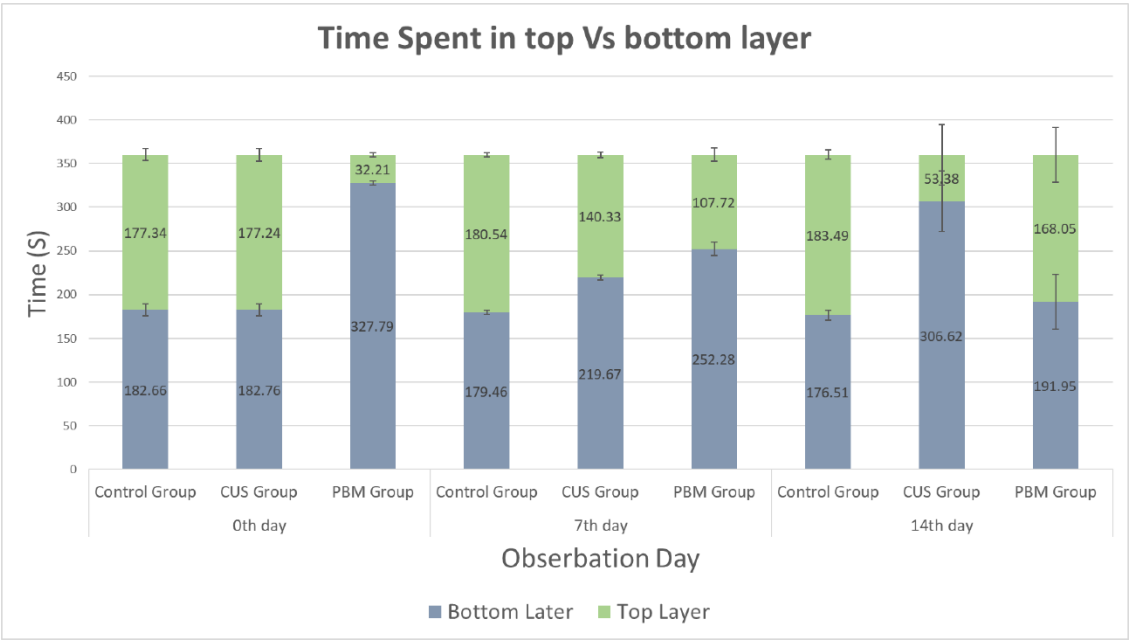

Figure 334: Plot for comparing average freezing episodes of different groups on different days of observation.

This is clear evidence that the fishes undergoing the CUS protocol are getting depressed. In the case of the PBM treatment group initially the time spent in the bottom region is significantly more than the top. As the treatment proceeds by the end of the 15th day we can clearly observe that the fish spend equal amount of time in both the zones.

Concluding the results, after the successful fabrication of the device we tested the device in a pilot study on the animal model of Zebra Fish. All the parameters which were taken for analyzing the behavior successfully indicated the positive healing effect of the PBM and the PBM device in animal model.

Chapter 5: Conclusion

5.1 Summery

Design and Development:

- Brain PBM device designed for depression treatment via photobiomodulation therapy.
- Extensive literature review on brain disorders, focusing on major depressive disorders and biological causes.
- Meticulous fabrication of the device including head unit and pocket unit using 3D printing and custom PCB design.
- Integration with a mobile app for convenient control and monitoring of therapy sessions.

Validation and Pilot Study:

- Pilot study conducted using a zebrafish model subjected to chronic unpredictable stress to induce depression-like symptoms.
- Behavioral analysis using ANY-MAZE software showed promising results:
- Improved exploratory behavior.
- Reduction in freezing episodes.

5.2 Conclusion

We successfully designed, developed, and fabricated a point of care device for treating Affective Disorders like depression and anxiety with a novel and non-invasive approach of photobiomodulation. With the animal model validation, we are sure about the therapeutic effect of NIR 830nm LED based device for curing depression and anxiety.

Chapter 6: Future Scope

The main aim of the device was to make a portable and affordable solution against affective disorder. Due to non-permit of ethical clearance a pilot study on animal model was conducted. With the future advancement of the device, we will go for clinical trials after the ethical clearance is granted.

Future work involves making market ready device with standard protocol of treatment, miniaturization and exploring the effect of PBM on other brain and neurodegenerative disorders.

REFERENCES

[1] Association, A. P. (2013). Diagnostic and statistical manual of mental disorders . *American Psychiatric Association*.

[2] Cacciatore, T. W. (2014). The application of photobiomodulation to the improvement of mitochondrial dysfunction in Alzheimer's disease and other neurodegenerative diseases. *Frontiers in neuroscience*, DOI: 10.3389/fnins.2014.00044. .

[3] Carvalho, A. F. (2016). The course of depressive symptoms and prescribing patterns of antidepressants in bipolar disorder: A naturalistic study. *Psychopharmacology*. doi:DOI: 10.1007/s00213-016-4211-y

[4] Cassano, P. P. (2016). Henderson and Morris.

[5] Cassano, P. P. (2016). Henderson and Morris experiments on penetration of NIR light into the skull.

[6] Chen, S. W. (2018). The role of mitochondria-associated cytoskeleton proteins in the regulation of mitochondria dynamics. *Frontiers in cell and developmental biology*. doi:Frontiers in cell and developmental biology

[7] De Freitas, L. F. (2016). Dose–response effects of photobiomodulation on experimental model of osteoarthritis. . *Lasers in Medical Science*, DOI: 10.1007/s10103-016-1941-3.

[8] De Freitas, L. F. (2016). Dose–response effects of photobiomodulation on experimental model of osteoarthritis. . *Lasers in Medical Science,*, DOI: 10.1007/s10103-016-1941-3.

[9] Demidova-Rice, T. N. (2010). Photobiomodulation therapy (PBMT) in wound healing: . *A review. Journal of Wound Care,*, DOI: 10.12968/jowc.2010.19.5.47704.

[10] Eells, J. T.-R. (2003). Therapeutic photobiomodulation for methanol-induced retinal toxicity. *Proceedings of the National Academy of Sciences*, DOI: 10.1073/pnas.0536854100. .

[11] Hamblin, M. (2016). Shining light on the head: Photobiomodulation for brain disorders. *BBA Clinical, 6*, 113-124. doi:10.1016/j.bbacli.2016.09.002

[12] Hamblin, M. R. (2016). Shining light on the head: Photobiomodulation for brain disorders. *BBA Clin (BBA Clinics, a journal from the Biochimica et Biophysica Acta family)*, 113-124. doi:10.1016/j.bbacli.2016.09.002

[13] Hamblin, M. R. (n.d.). Mechanisms and mitochondrial redox signaling in photobiomodulation. Photochemistry and photobiology.

[14] Hirschfeld, R. M. (2014). The mood disorder questionnaire: A simple, patient-rated screening instrument for bipolar disorder. . *Primary Care Companion to The Journal of Clinical Psychiatry*, 10.4088/PCC.v06n0301a.

[15] Karu, T. (2010). Mitochondrial signaling in mammalian cells activated by red and near-IR radiation. *Photochemistry and photobiology*. doi:DOI: 10.1111/j.1751-1097.2008.00490.x.

[16] Liebert, A. B. (2014). The use of low level light therapy in the treatment of androgenetic alopecia and female pattern hair loss. . *Journal of Dermatology & Dermatologic Surgery*, DOI: 10.1089/pho.2014.3896. .

[17] Luchiari, A. C. (2016). ebrafish (Danio rerio) responds to the animated image of a predator: Towards the development of an automated aversive task. *Neuroscience*, DOI: 10.1016/j.neuroscience.2016.09.031.

[18] Merikangas, K. R. (2011). Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. *Archives of general psychiatry,*, 241-251. doi:10.1001/archgenpsychiatry.2011.12.

[19] Naeser, M. A. (2014). Significant improvements in cognitive performance post-transcranial, red/near-infrared light-emitting diode treatments in chronic, mild traumatic brain injury: open-protocol study. . *Journal of neurotrauma*, DOI: 10.1089/neu.2013.3244.

[20] Porsolt, R. D. (1978). Behavioral despair in rats: A new model sensitive to antidepressant treatments. *European Journal of Pharmacology*, DOI: 10.1016/0014-2999(78)90118-8.

[21] Rush, A. J. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *American Journal of Psychiatry,*. doi:10.1176/ajp.2006.163.11.1905

[22] Tafur, J. &. (2008). Low-intensity light therapy: exploring the role of redox mechanisms. . *Photomedicine and laser surgery*, DOI: 10.1089/pho.2007.2172. .

[23] Wang, X. T.-L. (2017). Up-regulation of cerebral cytochrome-c-oxidase and hemodynamics by transcranial infrared laser stimulation: A broadband near-infrared spectroscopy study. *Journal of cerebral blood flow & metabolism*, DOI: 10.1177/0271678X17694185. .

APPENDIX

. Code for the ATMEGA328P

```
const int redLightPin = 7;
const int blueLightPin = 10;
const int speakerPin = 9;
const int Connection1 = 3;
int freqcy = 0;
int timeValue = 0;
int frequencyValue = 1;
int intensityValue = 255;

void setup()
{
    Serial.begin(9600);
    pinMode(redLightPin, OUTPUT);
    pinMode(blueLightPin, OUTPUT);
    pinMode(speakerPin, OUTPUT);
    pinMode(Connection1, OUTPUT);
    // Connecting Sound
    for (int i = 0; i < 3; i++)
    {
        digitalWrite(redLightPin, HIGH);
        tone(speakerPin, 700, 200);
        delay(200);
        digitalWrite(redLightPin, LOW);
        noTone(speakerPin);
        delay(200);
    }
    // Power On LED
    digitalWrite(redLightPin, HIGH);
}

void loop()
{
    if (Serial.available() > 0)
    {
        char receivedChar = Serial.read();

        // Convert the received character to an integer
        int mode = receivedChar - '0';

        // Perform actions based on the received mode
        switch (mode)
        {
```

```
        case 0:
            playConnectTone();
            break;
        case 1:
            blinkLed(5, 10); // Blink for 5 seconds at 10 Hz
            blinkLed(5, 40); // Blink for 5 seconds at 40 Hz
            break;
        case 2:
            beep(2);
            break;
        case 3:
            freqcy = 10;
            break;
        case 4:
            freqcy = 40;
            break;
        case 5:
            freqcy = 100;
            break;
        case 6:
            blinkLed(300, freqcy);
            break;
        case 7:
            blinkLed(600, freqcy);
            break;
        case 8:
            blinkLed(1200, freqcy);
            break;
        case 9:
            blinkLed(1800, freqcy);
            break;
        case 10:
            if (Serial.available() > 0)
            {                                                       //
Check if data is available to read
                String receivedData = Serial.readStringUntil('\n'); //
Read the received data until newline character

                // Tokenize the received data based on commas
                char *token = strtok(const_cast<char
*>(receivedData.c_str()), ",");
                timeValue = atoi(token); // Convert token to integer
                token = strtok(NULL, ",");
                frequencyValue = atoi(token); // Convert token to
integer
                token = strtok(NULL, ",");
```

```
                intensityValue = atoi(token); // Convert token to
integer

                // Flicker the Connection1 at the specified frequency,
intensity, and duration
                flickerConnection1(frequencyValue, intensityValue,
timeValue);
            }
        }
    }
}

void flickerConnection1(int frequency, int intensity, int duration)
{
    unsigned long previousMillis = 0;
    unsigned long interval = 1000 / frequency; // Calculate the
interval based on frequency

    unsigned long startTime = millis(); // Get the current time
    while ((millis() - startTime) < (duration * 60000))
    {                                          // Convert duration to
milliseconds
        unsigned long currentMillis = millis(); // Get the current time

        // Toggle the Connection1 based on the interval
        if (currentMillis - previousMillis >= interval)
        {
            previousMillis = currentMillis;  // Save the current time
            digitalWrite(Connection1, HIGH); // Turn on Connection1
            delayMicroseconds(intensity);    // Wait for intensity
microseconds
            digitalWrite(Connection1, LOW);  // Turn off Connection1
            delayMicroseconds(intensity);    // Wait for intensity
microseconds
        }
    }
}

// blink led function
void blinkLed(int durationSeconds, int frequencyHz)
{
    digitalWrite(blueLightPin, HIGH);
    int totalMillis = durationSeconds * 1000;
    int interval = 1000 / (2 * frequencyHz); // Calculate the interval
for the given frequency
```

```
    for (int elapsedMillis = 0; elapsedMillis < totalMillis;
elapsedMillis += interval * 2)
    {
        digitalWrite(Connection1, HIGH);
        delay(interval);
        digitalWrite(Connection1, LOW);
        delay(interval);
    }
    beep(5);
    digitalWrite(blueLightPin, LOW);
}
// blink led function  ends

void beep(int times)
{
    for (int i = 0; i < times; i++)
    {
        tone(speakerPin, 700, 200); // Beep for 200 milliseconds
        delay(300);                 // Pause between beeps
        noTone(speakerPin);
        delay(300);
    }
}

void playConnectTone()
{
    // Distinctive tone for connection
    for (int i = 0; i < 1; i++)
    {
        digitalWrite(redLightPin, LOW);
        digitalWrite(blueLightPin, HIGH);
        tone(speakerPin, 700, 200);
        delay(300);
        digitalWrite(redLightPin, HIGH);
        digitalWrite(blueLightPin, LOW);
        tone(speakerPin, 1400, 200);
        delay(300);
        digitalWrite(redLightPin, LOW);
        digitalWrite(blueLightPin, HIGH);
        tone(speakerPin, 2100, 200);
        delay(300);
        digitalWrite(redLightPin, HIGH);
        digitalWrite(blueLightPin, LOW);
        tone(speakerPin, 2800, 200);
        delay(300);
    }
```

```
    noTone(speakerPin); // Turn off the tone
}
```

Code for AT-Communication

```
1. 1. AT            // Check if the module is responding.
2. 2. AT+VERSION    // Get firmware version.
3. 3. AT+NAME="NewName"   // Set or get the Bluetooth device name.
4. 4. AT+ADDR?      // Get the Bluetooth address.
5. 5. AT+ROLE=x     // Set or get the role of the module (Master or
   Slave).
6. 6. AT+CMODE=x    // Set or get the connection mode.
7. 7. AT+RESET      // Restart the module.
8. 8. AT+UART=x,y   // Set or get the UART configuration (Baud rate, stop
   bits, parity).
9. 9. AT+PSWD="NewPassword"  // Set or get the password.
10.10. AT+ROLE?     // Query the current role of the module.
11.11. AT+INQM=x,y,z   // Set or get inquiry parameters.
12.12. AT+INQ       // Make the module discoverable.
```

Combined Data Of ANY-MAZE software

DAY 00 CUS PROTOCOL

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.051	0.048	0.057	0.062	0.055	0.058	0.049	0.064	0.046	0.063	0.0553	Average speed (m/s)
Total freezing episodes	0	0	0	0	0	0	0	0	0	0	0	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360		Total time mobile (S)
Time in the upper zone (S)	175.1	170.5	184.5	175.5	189.1	175.6	184.9	171.7	180.2	165.3	177.24	Time in the upper zone (S)
Time mobile in the upper zone (S)	174.6	169.9	184	174.8	188.6	175.1	184	171.2	179.5	164.8	176.65	Time mobile in the upper zone (S)
Time in the lower zone (S)	184.9	189.5	175.5	184.5	170.9	184.4	175.1	188.3	179.8	194.7	182.76	Time in the lower zone (S)
Time mobile in the lower zone (S)	184	188.6	174.6	183.6	170	183.5	174.2	187.4	178.9	193.8	181.86	Time mobile in the lower zone (S)

DAY 07 CUS PROTOCOL

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.044	0.046	0.045	0.05	0.049	0.048	0.043	0.048	0.047	0.045	0.0465	Average speed (m/s)
Total freezing episodes	0	1	0	0	0	1	0	0	1	0	0.3	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360		Total time mobile (S)
Time in the upper zone (S)	140.6	142.3	141.8	136.5	137.9	139.1	135.7	145.2	144.4	139.8	140.33	Time in the upper zone (S)
Time mobile in the upper zone (S)	137.1	139.9	139	132.9	134.5	135.6	130.2	139.7	138.8	134.3	136.2	Time mobile in the upper zone (S)
Time in the lower zone (S)	219.4	217.7	218.2	223.5	222.1	220.9	224.3	214.8	215.6	220.2	219.67	Time in the lower zone (S)
Time mobile in the lower zone (S)	218.7	172.7	217.5	223.1	221.4	175.9	223.6	214.3	170.6	219.5	205.73	Time mobile in the lower zone (S)

DAY 14 CUS PROTOCOL

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.039	0.038	0.036	0.035	0.037	0.037	0.038	0.034	0.04	0.034	0.0368	Average speed (m/s)
Total freezing episodes	4	0	3	4	1	5	3	3	2	4	2.9	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360		Total time mobile (S)
Time in the upper zone (S)	47.2	156.7	35.8	38.4	42.1	47.9	44.3	41.7	33.5	46.2	53.38	Time in the upper zone (S)
Time mobile in the upper zone (S)	2.1	24.6	25.1	18.6	1	1.6	23.9	21.9	20.3	1.2	14.03	Time mobile in the upper zone (S)
Time in the lower zone (S)	312.8	203.3	324.2	321.6	317.9	312.1	315.7	318.3	326.5	313.8	306.62	Time in the lower zone (S)
Time mobile in the lower zone (S)	130.7	178.7	164.1	123	271.9	85.5	156.8	161.4	216.2	132.6	162.09	Time mobile in the lower zone (S)

DAY 00 NIR TREATMENT

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.041	0.039	0.036	0.041	0.038	0.037	0.04	0.035	0.034	0.043	0.0384	Average speed (m/s)
Total freezing episodes	3	2	3	4	2	5	3	3	5	4	3.4	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360	360	Total time mobile (S)
Time in the upper zone (S)	30.2	29.8	32.1	34.5	31.7	36.8	28.9	35.2	33.6	29.3	32.21	Time in the upper zone (S)
Time mobile in the upper zone (S)	20.4	21.1	27.4	28.1	13.8	0.5	21.7	27.7	6	3.7	17.04	Time mobile in the upper zone (S)
Time in the lower zone (S)	329.8	330.2	327.9	325.5	328.3	323.2	331.1	324.8	326.4	330.7	327.79	Time in the lower zone (S)
Time mobile in the lower zone (S)	191.7	183.5	170.4	135.1	235.1	94.5	193.2	186.2	77.8	147.7	161.52	Time mobile in the lower zone (S)

DAY 07 NIR TREATMENT

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.049	0.048	0.054	0.053	0.045	0.037	0.051	0.047	0.052	0.046	0.0482	Average speed (m/s)
Total freezing episodes	2	2	1	2	2	4	2	2	1	2	2	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360	360	Total time mobile (S)
Time in the upper zone (S)	101.2	107.8	115.3	98.6	119.9	105.4	112.1	100.7	117.2	99	107.72	Time in the upper zone (S)
Time mobile in the upper zone (S)	100.7	107.1	108.8	92.1	113.4	104.9	111.6	100.2	115.7	98.5	105.3	Time mobile in the upper zone (S)
Time in the lower zone (S)	258.8	252.2	244.7	261.4	240.1	254.6	247.9	259.3	242.8	261	252.28	Time in the lower zone (S)
Time mobile in the lower zone (S)	168.8	162.2	199.7	171.4	150.1	74.6	157.9	169.3	197.8	171	162.28	Time mobile in the lower zone (S)

DAY 14 NIR TREATMENT

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.057	0.055	0.061	0.058	0.062	0.06	0.056	0.064	0.059	0.065	0.0597	Average speed (m/s)
Total freezing episodes	0	1	0	0	0	2	0	0	0	1	0.4	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360	360	Total time mobile (S)
Time in the upper zone (S)	183.2	107.8	179.8	192.3	188.7	105.4	172.6	191.9	177.2	181.6	168.05	Time in the upper zone (S)
Time mobile in the upper zone (S)	181.1	105.7	177.7	190.2	186.6	103.3	170.5	189.8	175.1	179.5	165.95	Time mobile in the upper zone (S)
Time in the lower zone (S)	176.8	252.2	180.2	167.7	171.3	254.6	187.4	168.1	182.8	178.4	191.95	Time in the lower zone (S)
Time mobile in the lower zone (S)	156.4	160.8	178.7	165.7	170.3	164.6	183.7	160.3	175.3	133.4	164.92	Time mobile in the lower zone (S)

DAY 00 CONTROL READING

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.05	0.045	0.056	0.059	0.054	0.057	0.048	0.062	0.043	0.061	0.0535	Average speed (m/s)
Total freezing episodes	0	0	0	0	0	0	0	0	0	0	0	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360	360	Total time mobile (S)
Time in the upper zone (S)	175.9	170.4	184.3	175.9	188.7	176.3	184	171.2	180.9	165.8	177.34	Time in the upper zone (S)
Time mobile in the upper zone (S)	174.2	168.7	182.6	174.2	187	174.6	182.3	169.5	179.2	164.1	175.64	Time mobile in the upper zone (S)
Time in the lower zone (S)	184.1	189.6	175.7	184.1	171.3	183.7	176	188.8	179.1	194.2	182.66	Time in the lower zone (S)
Time mobile in the lower zone (S)	185.8	180.7	177.4	185.8	167.6	185.4	177.7	190.5	169.4	195.9	181.62	Time mobile in the lower zone (S)

DAY 07 CONTROL READING

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.061	0.058	0.057	0.064	0.055	0.063	0.06	0.062	0.056	0.059	0.0595	Average speed (m/s)
Total freezing episodes	0	0	0	0	0	0	0	0	0	0	0	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360	360	Total time mobile (S)
Time in the upper zone (S)	178.2	181.5	183.7	180.1	177.9	182.4	179.6	184.3	176.8	180.9	180.54	Time in the upper zone (S)
Time mobile in the upper zone (S)	177.7	181	183.2	179.6	177.4	181.9	179.1	183.8	176.3	180.4	180.04	Time mobile in the upper zone (S)
Time in the lower zone (S)	181.8	178.5	176.3	179.9	182.1	177.6	180.4	175.7	183.2	179.1	179.46	Time in the lower zone (S)
Time mobile in the lower zone (S)	181.1	177.8	175.6	179.2	181.4	176.9	179.7	175	182.5	178.4	178.76	Time mobile in the lower zone (S)

DAY 14 CONTROL READING

PARAMETERS	FISH 1	FISH 2	FISH 3	FISH 4	FISH 5	FISH 6	FISH 7	FISH 8	FISH 9	FISH 10	AVG FISH	
Total Test duration (S)	360	360	360	360	360	360	360	360	360	360		Total Test duration (S)
Average speed (m/s)	0.057	0.055	0.061	0.058	0.062	0.06	0.056	0.064	0.059	0.065	0.0597	Average speed (m/s)
Total freezing episodes	0	0	0	0	0	0	0	0	0	0	0	Total freezing episodes
Total time mobile (S)	360	360	360	360	360	360	360	360	360	360	360	Total time mobile (S)
Time in the upper zone (S)	179.7	191.2	185.3	188.5	180.8	177.6	183.4	176.9	192.1	179.4	183.49	Time in the upper zone (S)
Time mobile in the upper zone (S)	177.6	189.1	183.2	186.4	178.7	175.5	181.3	174.8	190	177.3	181.39	Time mobile in the upper zone (S)
Time in the lower zone (S)	180.3	168.8	174.7	171.5	179.2	182.4	176.6	183.1	167.9	180.6	176.51	Time in the lower zone (S)
Time mobile in the lower zone (S)	179.6	168.1	174	170.8	178.5	181.7	175.9	182.4	167.2	179.9	175.81	Time mobile in the lower zone (S)