Implantable Light Source Development

BME Design 200/300

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Abstract

The field of optogenetics has been growing in recent years, as its applications are useful in understanding and controlling the behavior of different cell types and neurons. Using optogenetics requires the target cells to be exposed to a light with a specific wavelength that will allow the cell to express fluorescent properties. Numerous optical stimulation tools have been developed to trigger this response in the proteins of the cell membranes, however there have been issues with the light not penetrating deep into the target tissues to photoconvert the cells, and fail to reach large areas with the hindrance of other factors in the body, such as organs or bones. To correct these issues, the "LED Mat" design was created, which consists of 4 LEDs connected in parallel sitting on a board and incorporated into biomaterial. The wavelength and intensity of the "LED Mat" could be controlled by the user, which allows for easier and safer photoconversion of the cells in the mice. The device is also programmed to turn on and off for 30 second intervals. The team selected PDMS (Polydimethylsiloxane) to enclose the design for biocompatibility. After analyzing the components of the light, the programmed LED emits 470 nm mainly. However, after covered in PDMS, there was also a second peak at 520 nm relatively weaker than 470 nm for LED. Since 470 nm still has stronger light intensity and 520 nm does not damage the cells, the design could be potentially effective for photoconverting the cells.

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I. Introduction

Tuberculosis (TB) is a potentially serious infectious disease that mainly affects the lungs. Even though most infections do not have symptoms, about 10% of those latent infections progress to an active disease which kills about half of those infected [1]. To cure tuberculosis in humans, many research projects have been carried out to find ways to alter immune cell functions in mice to understand inflammatory responses to diseases in the brain and lungs. Currently, researchers are using mice as models, as they have strong immune systems, and their genetic, biological and behavior characteristics closely resemble those of humans. A widely used technique to alter immune response is optogenetics, which can control and monitor the activities of individual neurons in living tissue, even within freely-moving animals, and to precisely measure the manipulation effects in real-time [1]. Nowadays, there is an increasing need to construct novel optogenetic implants by using appropriate engineering approaches. These implants should be able to achieve precise light emission, and to reliably deliver light to targeted areas. The implants should also be capable of being applied for multi-site (area) and multi-layer (depth) operations so that the light intensity can reach a bigger threshold. Over the past two decades, numerous optical stimulation tools have been developed, however they are hard to be employed into vivo applications since these tools are mainly based on either the utilization of exogenous cofactors or the expression of multiple proteins [2]. Issues have also arisen specifically when delivering light to photoconvert cells in the lung tissue of mice: insufficient light intensity when using a light with a small wavelength, the areas that the light can reach due to blockage by the ribs of the mice, the depth the light can penetrate into the lung tissue, and bleeding/bruising is observed occasionally which obscures the light. [3]. Therefore, a device is needed to eliminate these issues.

The brainstorming process for the design began with looking to existing designs for inspiration. There are many LED devices and processes that already exist to photoconvert cells in KikGR33 mice. The current design the client uses inspired parts of the design created. Dr. Sandor and his lab members use fiber optics to photoconvert the cells in the lungs of the mice. The lab is currently using approximately 1000 mW 405 nm light. Since light is pivotal in the photoconversion process, a few fiber optic cable adjustments have been already made: the conversion area has been increased by a higher NA (numerical aperture from 0.22 to 0.4) to increase the cone of emission from 25° to 45°**,** and the output intensity has been increased by increased cable width (from 0.69 to 0.87 mm) and decreased exposed fiber (Figure 1). The fiber optic wire is inserted into the mice using a syringe and needle, while the mice are stabilized on an intubation stand [3]. There are other competing designs that are being used in the optogenics field; however, some of them are very expensive and Dr. Sandor asked for a design that could either be fully sterilized or cheap enough to allow for multiple uses. One design on the market uses principles from Faraday's law to induce a current to power the LED light in the mice, which allows the device to be wireless [4]. A different design proposes sandwiching a micro LED light

between a backside reflector and a frontside microlens: the backside reflector collects the light that leaks from the bottom of the LED chip, and the microlens aligns the light rays so they reach deeper into the tissue [5].

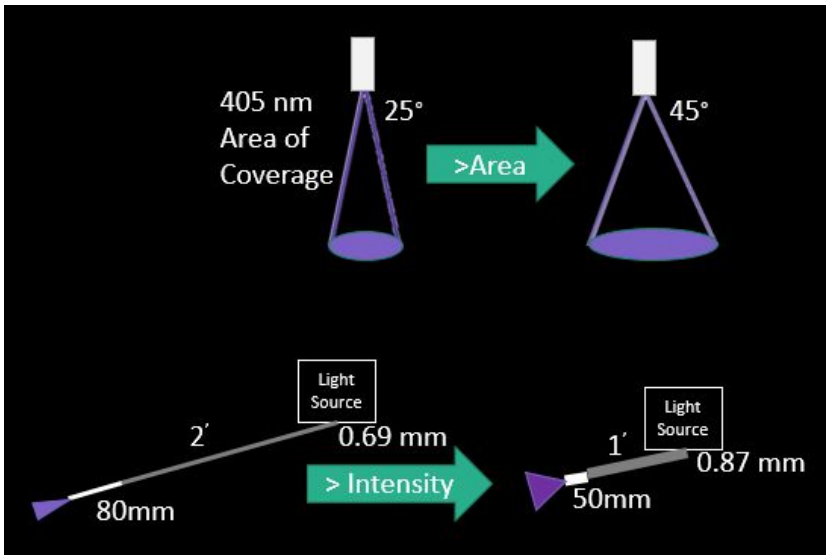

Figure 1: Fiber optic cable adjustments have been made

Dr. Sandor's previous method of photoconversion was inefficient, and only affected small areas; not all photoconversion sites can be found. Therefore, a new device needed to be designed to allow for photoconversion of larger areas for 20 minutes with appropriate wavelength and greater intensity. The design must fit in the mice and safely photoconvert cells without causing harm to the mice.

II. Background

Optogenetics is a technique which involves the use of different wavelengths of light to control living cell activities. Photoactivatable fluorescent proteins have been developed whose fluorescent properties change based on their exposure to light [6]. Exposing cells that have been altered to include these proteins in their cell membranes to certain wavelengths of light opens these protein channels and allows the influx of different types of ions into the cell [3]. This is a useful imaging tool, as it allows the examination of living cells at nanometer resolution [6]. Optogenetics also allows the tagging of the fluorescent cells, which is useful to see the path that the cells have traveled [8]. The clients, Dr. Sandor and his lab team, are taking advantage of this particular benefit of optogenetics to study the immune response to tuberculosis in the lungs of kikGR33 mice: the travel path of the dendritic cells are examined as they migrate to the granuloma cells, showing which dendritic cells are not working correctly [7]. Granuloma cells in the left lung of the mice are photo converted to emit the color red when exposed to a light with a

wavelength of 470 nanometers, which can then be easily distinguished from the green-dyed systematic cells of the lung tissue in the mice (Figure 2)[8].

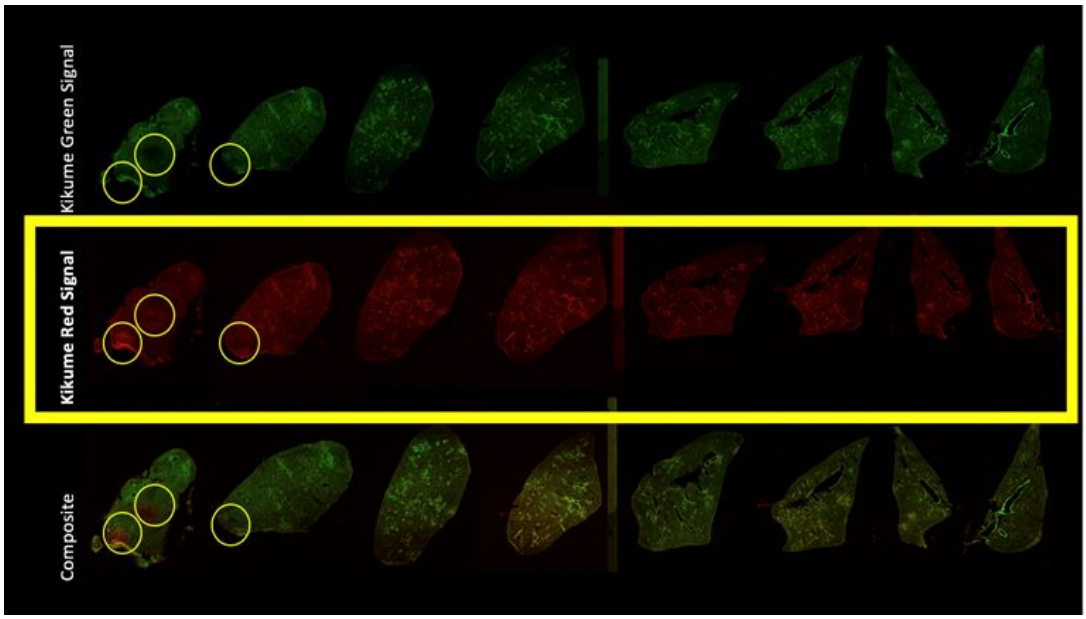

Figure 2: Circled areas are areas of photoconversion

Biocompatible materials are materials that do not cause a harmful reaction when implanted inside of a living animal. Some examples of biocompatible materials include steel, bioceramics, and PDMS (Polydimethylsiloxane) [8]. They vary from rigid and opaque to flexible and transparent. For the purposes of an implantable light, a transparent and flexible biomaterial is needed. PDMS fills this need perfectly as it comes in a liquid form and does not become solid until treated with UV light. This allows it to form around various structures. In addition, PDMS is very transparent, which lets light shine through it [9].

The client is in need of a more efficient method of photoconverting the granuloma cells in the lung tissue. The device must have a size of approximately 1 cm^2 with a broad light source range able to penetrate deep into the organs of the mice (> 95mW/cm^2) and be automated to allow the lights to be turned on and off every 30 seconds. It also needs to have a wavelength of 470nm without producing UV rays or excess heat, which would harm the cells in the mice (refer to *Appendix I*).

III. Preliminary Designs

1) "Disco Bulb":

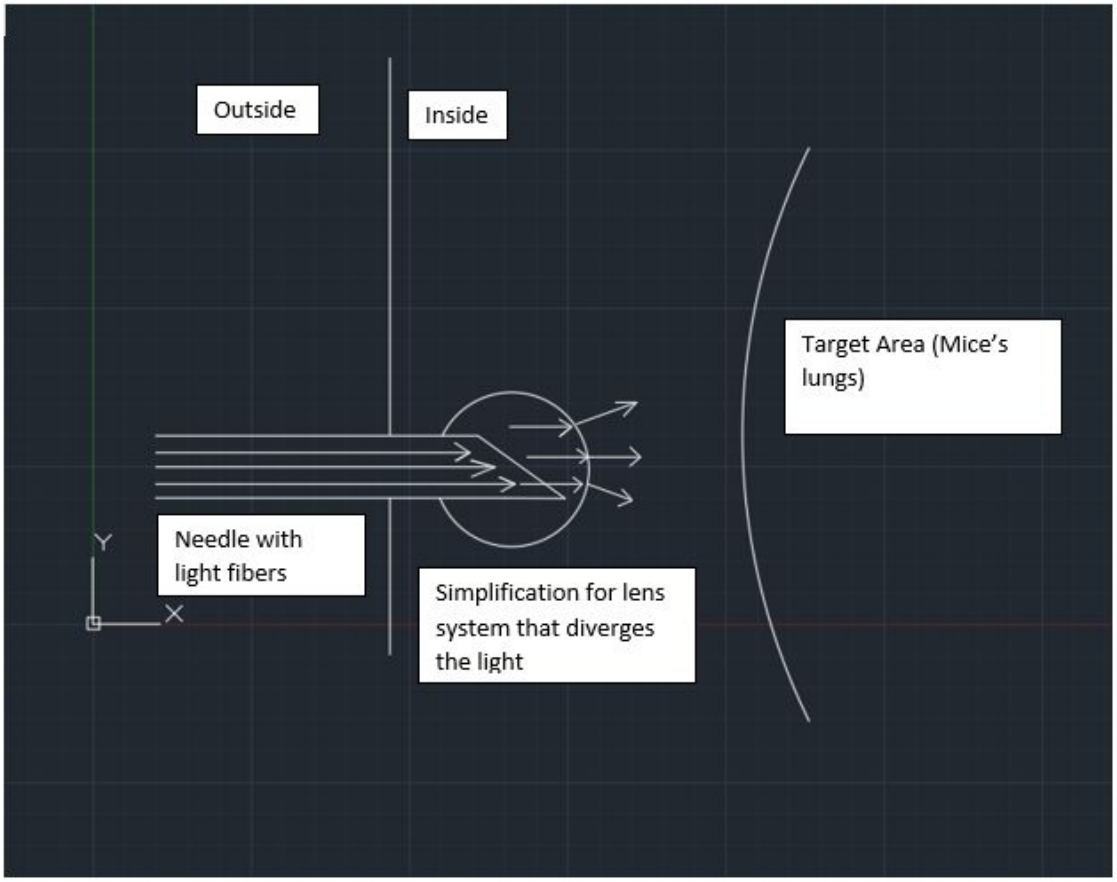

Figure 3: AutoCAD-drawn "Disco Bulb"

This design utilizes a lens system to diverge the light delivered from the light fibers. The light fiber is inside the needle and the lens system is set at the tip of the needle. When the light, shown parallel to each other on figure 1, reaches the tip of the needle that is being implanted, the light will be diverged into various directions to cover more areas on the lungs. This design allows our client to adjust the light source from the outside of the mice body by using microcontrollers and light source directly instead of inserting LEDs into mice as they might be hard to control when implanted. The client does not have to worry about the type of the light source as long as the light delivered has enough intensity.

This feature also offers the clients more convenience to switch the wavelength of the light without further damage to the mice. However, manufacturing the lens could be a challenge for our group based on the dimensions of the lens system. Moreover, the diverging effect of the lens may compromise the strength of the light which may not create enough trigger for depolarization of the neurons.

2) Magnetic Fan:

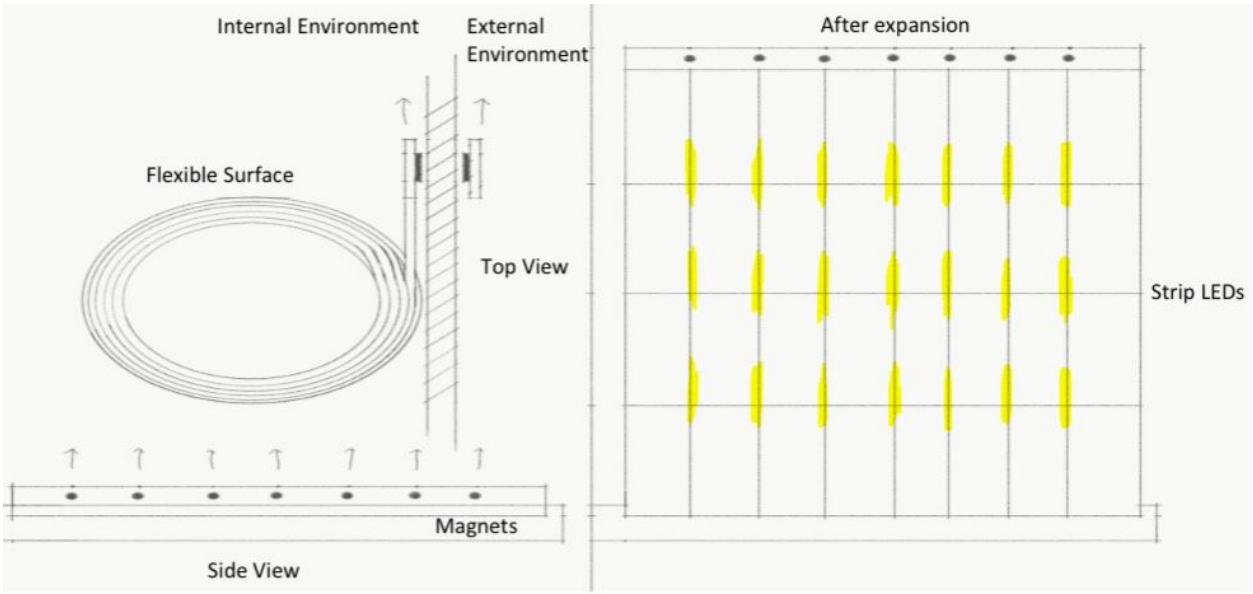

Figure 4: Illustration of the design working principle. Left: side view and top view after implantation. Right: Strip LEDs (yellow) after expansion

This design shown in figure 2 most resembles the working principle of a curtain. The device is similar to a scroll before implantation and the end of the scroll has magnetic attached. Strip LEDs are integrated in the design shown in yellow. The material of the design is biocompatible with enough flexibility to be wrapped into a cylinder.

After the implantation, clients could use a plate that is able to generate enough magnetic forces to expand the “fan”implanted into the mice from the outside, which could reduce the harm by implantation. The expansion inside the body allows the LEDs to wrap around the ribcage which could maximize the area lightened.

The drawback of the design is the complexity and the maintenance of the product. It is more complicated and the maintenance process might also be complex. The embedded magnetic control system also makes it hard for magnet selections.

3) **Bioluminescent Fluid:**

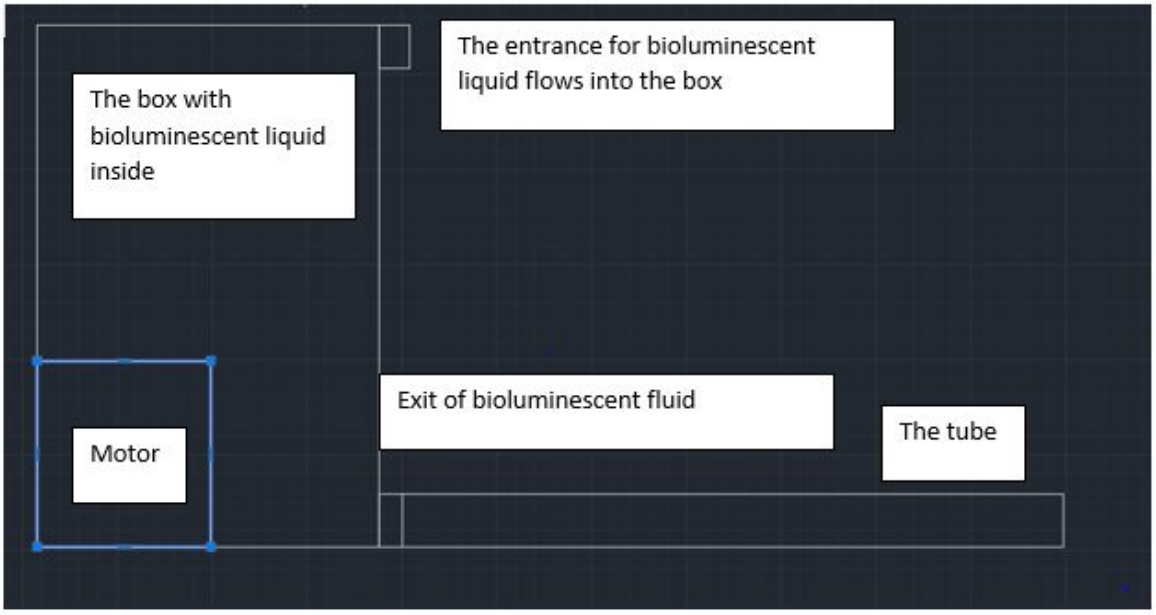

Figure 5: Top view of the design which shows the box for circulating the bioluminescent fluid

This design relies on a biocompatible tube filled with bioluminescent fluid. The light is delivered by the implantation of the tube near the lung. The bioluminescent bacteria are kept inside the transparent tube which allows the light to be transferred to the lung while avoiding contamination to the cells of the mice.

The box's function is to circulate the bioluminescent fluid once the tube enters the mice and leaves the mice. The tube could be plugged into the entrance labeled in the drawing. The motor inside the box could then change the air pressure to generate the flow of the liquid which can drive the bioluminescent fluid in one direction.

The reason why our team came up with this design is that our team is concerned with the overheating of the device which could cause harm to the cells of the mice. The use of bioluminescent fluid has the potential to be the solution because most types have less than 20% of the energy being converted to heat [10].

However, this device is not reusable compared to other designs so it leads to low economic efficiency: the light is generated by bacteria which could die at a fast rate. In this case, researchers might need to replace the liquid often to ensure the light intensity. Also, the design lacks a mechanism for switching the light on and off. Furthermore, the biggest challenge might be the selection of bioluminescent liquid as only specific types of bacteria in the liquid can

generate light at a wavelength we desire. Therefore, this design may potentially cost much more money than other designs and needs more future work.

4) **LED Mat:**

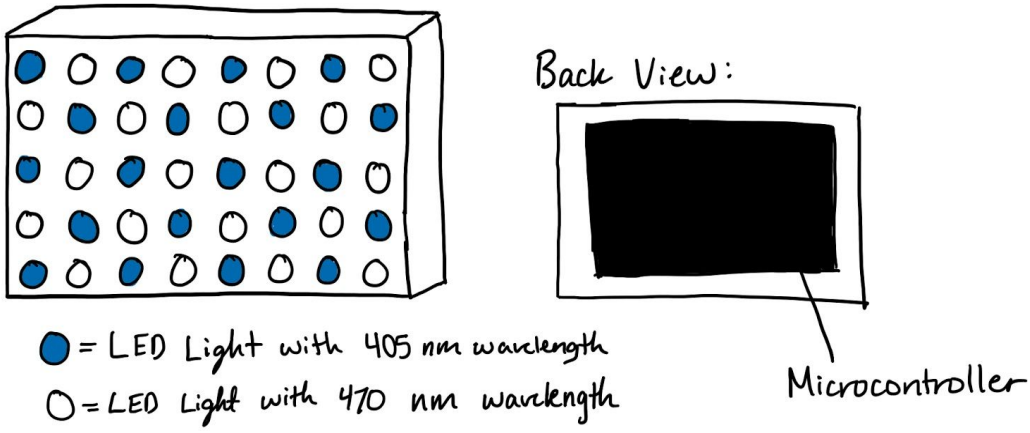

Figure 6: Illustration of the LED Mat. LEDs with different wavelengths are embedded on a biocompatible mat with a microcontroller/ microprocessor attached as shown in the back view.

This design consists of several lights integrated into a flexible, three dimensional, biocompatible mat. The LED lights will be alternated across the rows and down the columns between lights with a 405 nm wavelength and 470 nm wavelength. Behind the lights, but inside of the mat, will be a microcontroller that will allow the device to be automated: every 30 seconds, the lights will alternate between the 405 nm wavelength and 470 nm wavelength.

This design allows the client to easily switch between the desired wavelengths, and does not require a human operator to do so. The flexible material of the device will allow the device to wrap around the ribcage and not cause too much discomfort with the mice. The lights occupy a large surface area, allowing a broad scope of light to reach the lung tissue and therefore penetrate deeper into the tissue.

However, this device can be bulky, raising a concern that it will not fit comfortably and easily in the mouse's abdomen. Also, the procedure to implant the device is invasive, as it might require the operator to cut a slit in the mouse's side to slide the device under the mouse's skin and over the ribcage. There is also concern that there will be a level of heat produced by this device that could be a danger to the cells.

IV. Preliminary Design Evaluation

Design Matrix

Criteria (weight)	**Magnetic Fan**	**Disco Bulb**	**Bioluminescent Liquid**	**LED Mat**
Cost (5)	4	3	5	4
Safety (20)	6	16	12	16
Size (15)	10.5	12	7.5	10.5
Efficiency (25)	20	17.5	12.5	20
Ease of Use (10)	6	7	8	8
Feasibility (10)	4	7	2	7
Materials (15)	10.5	12	9	12
Total (100)	61	74.5	56	**77.5**

Table 1: Design matrix of four proposed designs. Different criteria have different weights as shown in the table. The highest total score is highlighted in yellow and that corresponding design is the team's focus this semester

Design Criteria:

Cost

Our cost is ranked as the lowest criteria, weighting 5%, because we have a high limit on our budget. As we ranked our designs, the lower the number, the less the cost would be for making that design. The cost of LED lights is relatively low, so we predict that the designs using this as a light source (the Magnetic Fan and LED Mat) will be relatively low. The Disco Bulb, which uses a light of the specified 405 nm and 470 nm wavelengths, includes a glass lense that will widen the range of the light projected. This design has the lowest predicted cost because the materials have a very low predicted cost. The Bioluminescent Liquid design has the greatest cost, as the glowing liquid, often used in surgeries, has a high predicted cost.

Safety

Safety was ranked as the second most important criteria with a weighting of 20%, because we need to keep the mouse alive. We decided that the less invasive the design was the safer it would be. That led to the Disco Bulb and LED mat being ranked the highest, as the Disco

Bulb uses a small needle and the LED mat just needs a tiny incision to be placed in the mice. The Bioluminescent Liquid was a close second as the tube the liquid id in will not bump into any organs, but it does require a relatively big incision to be placed into the mice. Our least safe was the magnetic fan because getting it into the mice would be invasive and once in the moving of the lights could cause the design to run into organs and blood vessels.

Size

Size was ranked in the middle, weighting 15%, because it is important to try and make our design small, but if it is a little bigger it just needs to be flexible. Our highest rated design was the Disco Bulb because it uses a small syringe. After the Disco Bulb we had a tie with the Magnetic Fan and the LED mat. Since both will be using a thin sheet with LED's on it we figured the size would be similar to each other. Our lowest rank for size was the Bioluminescent Liquid as this design will use a tube to hold the liquid and its size would be large compared to our other designs.

Efficiency

Efficiency was our highest ranked criteria at a weighting of 25% because our clients main problem with their product right now is that it is inefficient. We decided that our LED mat and our Magnetic fan would be the two most efficient designs. We made this decision based off the fact that both could have LEDs of 405 nm and 470 nm wavelength and would be able to cover a larger area better and faster than our other designs. Close behind these designs was the Disco Bulb, since it could spread the light out over a large area but we worried that refracting the light would cause inconsistent spreading of the light. Our lowest ranked was the Bioluminescent Liquid because it would be hard for it to cover a large area quickly and the light of the liquid may not be intense enough.

Ease of use

Ease of use is weighted to be 10% of the total design score, because our client said it would be nice if the led lights are wirelessly controlled. This is not one of their main concerns, but it is a feature that would make the design easier to use. The LED mat and bioluminescent liquid designs scored the highest in this category, because they are the easiest to use once implemented. The LED mat would be wireless, and the bioluminescent liquid design would be very easy to use once it is set up in the mice. The magnetic fan design scored the lowest in this category, because it requires more involvement to get the light to penetrate the lungs of the mice. A person would have to physically move the fan across the mice to expose the lungs to the light, which makes it harder to use.

Feasibility

Feasibility is weighted to be 10% of the total design score, because it is important to consider how feasible it is for us to create each design with the amount of time and resources that we have. The LED mat and the disco bulb scored the highest in feasibility, because there is a higher probability that we can get the materials required for these designs. Our bioluminescent

design scored the lowest in this category, because the materials needed for this design are very hard to find and they are expensive. The process to implement this device in the mice would be more complicated than our other designs.

Materials

Materials is weighted to be 15% of the total design score, because there are a lot of factors that we need to take into account when choosing materials for our design. The materials that we choose need to be durable, not too expensive, and easy to obtain. The materials need to be able to be inside the mice without their bodies rejecting it. The disco ball and the LED mat designs scored the highest in this category, because the materials needed for these designs are more easily accessible. The bioluminescent liquid design scored the lowest in materials, because the materials needed are very expensive and harder for us to obtain.

Final Design:

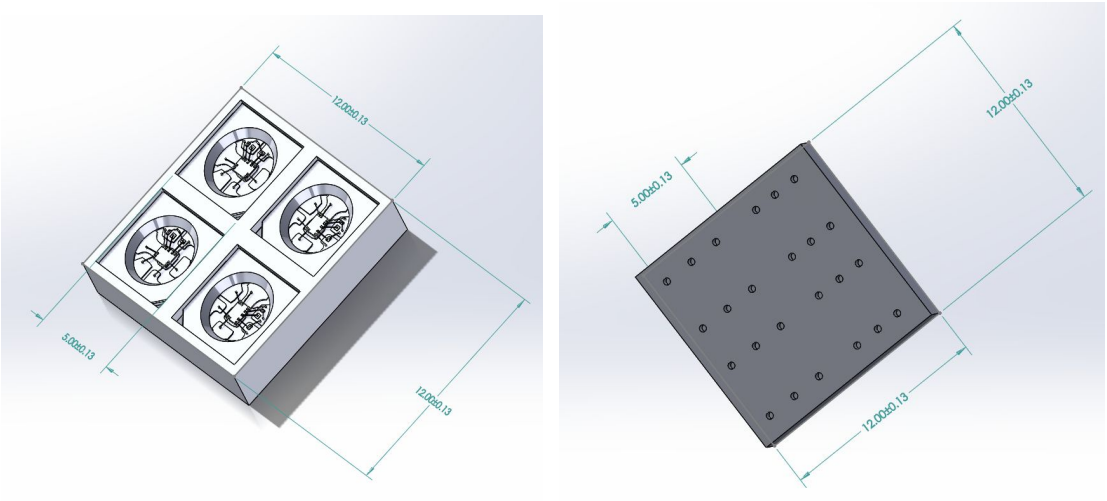

Figure 7: Top view and bottom view of LED mat

The team decided to use four LEDs that deliver light at wavelength at 470 nm since the team agreed that four LEDs are able to deliver light that is intense enough to light up 1cm^2 of cells on the mice lung. The dimension of an individual LED is 5mm x 5mm x 1.4mm and the team decided to leave 1mm between two LEDs and 0.5mm from the LED to the edge of the mat. The thickness of the mat is designed to be 2mm with 1.4mm being the thickness of the LED and 0.6mm for the space to solder wires on the pins. Therefore, the dimension of our designed LED mat to be implanted into mice is 12mm x 12mm x 2mm. Each LED has six pins on it so the team will drill six holes for each LED (24 holes in total with 0.6mm in depth) for the wires to pass through. The material of the mat the team chose is steel as it can act as heat sinker to absorb heat generated from the LEDs. As for the biocompatibility of our device, the team planned to use

PDMS because PDMS is transparent, flexible, and biocompatible. The LED mat will be enclosed in PDMS in our final phase of fabrication.

V. Development Process

Fabricated Prototype:

Our fabricated prototype is shown below in figure 8. The team loaded and saved the code onto Arduino microcontroller. The microcontroller powered by laptop or other electronic devices can output 5V to the LED. Four LEDs are connected in parallel by the circuit built on the breadboard. Four pins on the LEDs are used. Pin 1 is for data input, getting instruction from the codes written; Pin 2 is for Clock Input, synchronizing the devices and setting the rhythm of LEDs' on and off; Pin 3 is for grounding our LED and Pin 4 is VCC+ which is for powering the LED by 5V from the microcontroller.

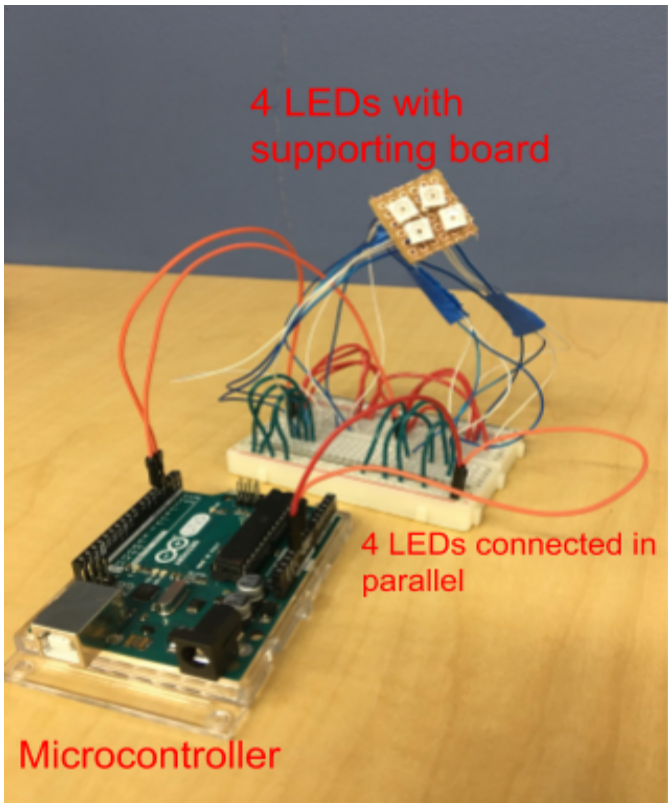

Figure 8: Fabricated prototype

Materials:

A main part of the design is the light source used since the light is what causes the photoconversion to occur. The LEDs need to be very small in order to fit inside the mice, and

they also needed to emit 470nm light. The team purchased Adafruit LEDs (APA102 5050 RGB LED)

Figure 9: APA102 5050 RGB LED

The LEDs are connected to the Arduino by a breadboard and wires obtained from the Makerspace. The Arduino is a microcontroller that controls the LEDs, which is important for the design because the client expects that the lights to be programmed to be on/off for certain time periods. The microcontroller could output different voltages to pins of the LEDs which could then operate as . The team has chosen Arduino Uno because the team is more familiarized with programming and the cost is affordable.

The wires were planned to be soldered onto the pins via SMD soldering paste but this method did not work because the LEDs are tiny; an oven is needed to solder the wires but it is hard to keep the wires in place in the oven. Instead, the team tried to hand-solder the wires onto the LED pins with a soldering iron and tin.

Once soldered onto the LEDs, the wires would be threaded through a circuit board to provide support for the LEDs and prevent the soldered wires from falling off. The plan was for the LEDs to be mounted to a steel sheet, which would act as a heat sink reducing the heat released in the mice. The prototype does not include this detail yet, but could be added in future work on the device [8].

The biocompatible material the team chose, Polydimethylsiloxane (PDMS), was acquired from McClean Lab in Department of Biomedical Engineering and was tested to determine the effect of the PDMS on the wavelength and intensity of the light. PDMS is a good biocompatible material so the PDMS-enclosed device will not affect the mice as much when inserted into their body.

Methods:

Due to time constraint, the team was not able to laser cut our purchased steel sheet into 12mm x 12mm x 2mm pieces for our prototype. Instead, we used a 12mm x 12mm circuit board as an alternative to support the LEDs structurally. The wires were soldered on the LED pins and one end of each wire passes through the holes on the circuit board so that the LEDs can sit on it. Then, the team used hot glue to seal the other side of the hole on the circuit board so that the LEDs were stabilized further. The breadboard was used to extend the wires and connect the wires to Arduino microcontroller. The on and off and delay time for each status were coded in

Arduino Uno and the team could change the brightness and wavelength of the LED to test the effectiveness of the device. For the testing process, the team borrowed Ocean Optics Spectrometer USB 2000+ and set up our testing in a dark environment. There were two groups in the testing phase: one control group to test the intensity and wavelength of the light and one experimental group to test whether the PDMS enclosing the device would affect the device's effectiveness.

Testing:

First we checked if the circuit would work outside the body. The test is mainly on the basic function of the circuit. Our team tested whether the light could be turned on and off by itself using code from the microcontroller.

The intensity and wavelength of the LED with PDMS covering it and without PDMS was tested by using the Ocean Optics USB 2000+ spectrometer. The LED was programmed on Arduino Uno to have 80% brightness for our testing purpose.To avoid the noise in the data caused by ambient lights, our team set the experiment in a closed cabinet which blocked most of the light source in the room. USB 2000+ measured the light intensity by the counts which is the number of times the photon was recorded at a certain wavelength within 100ms by default.

The team tested the LED light intensity with PDMS put in between the light source and the spectrometer to see whether PDMS is a possible solution. The distance between the light and spectrometer is the same in two sets of experiment.

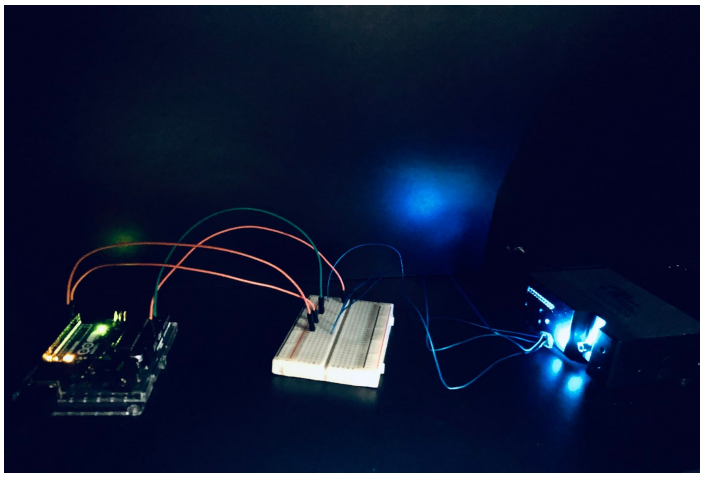

Figure 10: Spectrometer and testing setup

The graphed data from the spectrometer was then analyzed in Matlab. The results of these tests will tell us if the LED reaches the intensity of a minimum of 95 mW/cm^2 , and is at

the wavelength of 470 nm. The tests will also help to evaluate PDMS as a potentially viable biomaterial to maintain our light delivery.

The light was measured in 15 minutes every time in the dark environment for consistency.

VI. Results

The data from our testing gave positive results. The LED without PDMS covering it had a significant peak at 470 nm in wavelength and no other significant peaks with X-axis being the measured wavelength and Y-axis the counts.

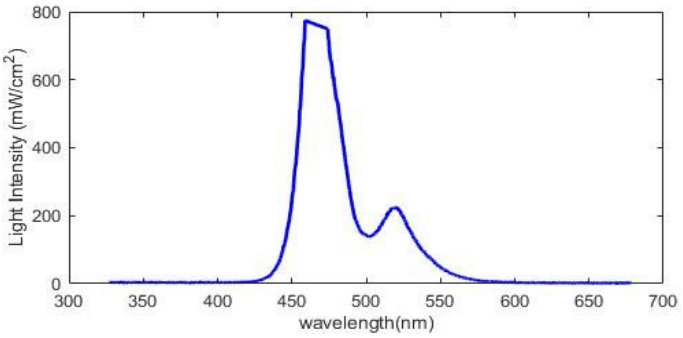

Figure 11: Light intensity without PDMS covered

The light intensity specified by our clients is in mW/cm^2, and USB 2000+ gives a light intensity measured in counts every 100ms. Every count of the photon energy is calculated with $h * c/\lambda$ where h is planck constant, c is the velocity of light, and λ is the wavelength evaluated. Since the counts is measured within 100ms, counts in one second is 10 times more than the counts in 100 ms. USB2000+ has light sensitive array with 2048 pixels which is 14μm x 200μm [6]. Then the light intensity within certain area is calculated by the light energy divided by the pixel area.

$$E = counts * \frac{1s}{100ms} * \frac{h * c}{\lambda} \qquad \text{Equation (1)}$$

$$light\ intensity\ per\ area = \frac{E}{Area} \qquad \text{Equation (2)}$$

By using these 2 equations, the team could convert the test data into the light intensity units specified by the clients. During the testing, the light measured was oversaturated as the intensity of the light hit 800 mW/cm^2, the maximum intensity that can be detected by the spectrometer. The results for the LED covered in PDMS were similar. The intensity was still at a maximum, which shows the PDMS does not impede the light significantly. The wavelength still had a large peak at 470 nm, but there was a second significant peak at 530 nm.

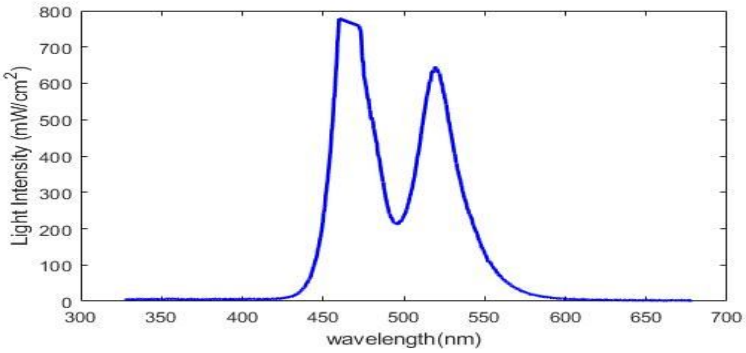

Figure 12: Light intensity with PDMS covered

	470 nm Peak Strength average(mW/cm^2)	520nm Peak Strength average(mW/cm^2)
No PDMS	774.5796 (oversaturated)	225.9
With PDMS	774.5796 (oversaturated)	646.6

Table 2: Maximum light intensity (mW/cm^2) in the two experiments

VII. Discussion

Light delivery is crucial for the experimentation of optogenetics. For optogenetics research on neural transduction, accuracy is needed and suggested light delivery system is designed to trigger as accurate area as possible. Because of this, most of light delivery system resembles the shape of a needle. However, our clients utilize optogenetics as a tool for studying immune response which demands the ability of light source to trigger area as big as possible. That is where the LED Mat design idea comes in, as it has the potential to photoconvert a large area with sufficient intensity.

The results show that the LED used is close to perfect as it is small, it delivers intense light, and it emits light at the correct wavelength. In addition, the results show that PDMS has the potential to be a viable biomaterial to cover the LEDs to be implanted in mice. The biomaterial is needed because the LEDs will be inside a living animal, so minimizing the device's reaction with biological fluids is necessary.

The results also show a second peak of light strength at about 520 nm when the light is covered by PDMS. One possibility may be that the PDMS is fluorescent [11]; there would be

unexpected wavelength peak. However, it is not a concern for the optogenetics use since 520nm does not fall under the UV range and does not damage the cells. Also, the intensity of light at 470nm is sufficient enough to trigger the cells so the LED mat is an effective device.

Moreover, some difficulties in manufacturing the design were encountered. Because the size of the LED is small, the pins on the LED can easily break without the support of the circuit board. Therefore the material selection of the wires and soldering techniques were thoroughly reconsidered. The problem was solved with soldering thinner wires with more flexibility and less radius.

The changes that need to be made based off of evaluating the results are creating a wireless device and thinning out the PDMS. Since our device currently would not be able to fit between the skin of a mouse and its rib cage, the first change needs to make the device thinner so it can fit. The most logical solution to this is making the device wireless, which would decrease its bulk significantly. The second change is thinning out the PDMS. The results show that the PDMS caused a second large peak in a wavelength we do not want to be a factor. The PDMS used in testing was thick, so a simple solution to this problem is making the PDMS thinner so that it disrupts the wavelength less.

Sources of error from our testing are the angle of the light from the LED and calibration of spectrometer. The spectrometer works by using a set of mirrors to reflect the light, so if our angle of the LED changed while running a test it may cause an less accurate reading in wavelength and/or intensity. Additionally the spectrometer we used had been calibrated many years ago, so the calibration could have been off causing the data it collected to be less accurate.

VIII. Conclusions

Optogenics combines optical and genetic methods to activate or inhibit certain protein channels. This technique is still relatively new and much work is being done to create optogenetic implants that can be used in multiple areas of the body and penetrate multiple layers of tissue that is being observed. The team's goal for this semester was to create an optogenetic light implant that helps study the bodies response to tuberculosis by photoconverting granulomas. Since tuberculosis is generally found in the lungs, our light must be able to shine on the lungs with the correct wavelength and intensity to photo-convert these granulomas. By doing this, Dr. Sandor and his team will be able to see how the body responds to the pathogen. In order to achieve this goal our design needs to be small and biocompatible.

LED mat is the implemented design for the project since it has better feasibility, safety, and easier material selection compared to the other preliminary designs. The design is still in developing phase, but based on our testing results we believe that it has the ability to trigger cellular responses over a large area. Further work still needs to be done, so that the mat can be enclosed in PDMS and fit between the skin and the rib cage of the mouse

IX. Future work

The next steps for our prototype are enclosing the LED Mat in PDMS, enabling wireless control and adjusting the shape of the mat. First, the team needs to cover the whole device in fresh PDMS and test the intensity and wavelength again to double check that PDMS is a viable solution. PDMS would also potentially allow us to get rid of the bulky board that is supporting the LEDs because PDMS will be able to provide the support and hold the soldering in together; also, the PDMS has the potential to absorb some heat. Future testing of heat generated by the LEDs is needed.

The team would also need to adjust the brightness of the light to attune autofluorescence effect of PDMS and have 520 nm light intensity to be less than 50% of the light intensity at 470 nm.

The team would be looking for ways to enable wireless control. This would help to eliminate the wires, which impede the mat from being able to go underneath the skin. This would also allow our client to control the light remotely and more compliance to experimental setup.

Lastly, the team will try to adjust the shape of the mat so that it fits between the rib cage and skin of the mouse. This would let our device slide under the skin easier and possibly allow for a larger area of photoconversion. Consequently, the team will test the device in vivo as our ultimate goal.

X. References

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Appendices

Appendix I: Product Design Specifications

Function:

The discovery of microbial opsin genes, which is a group of genes that enable the neuron to be activated by light, makes it possible to selectively control activation of neuron by light. Optogenetics is the study that combine genes and the emission of light (optics) together. Our client aims to study how tuberculosis could be treated by observing how the immune system responds to the infection. Our client does this by photoconverting granulomas in the lungs and seeing how they change overtime. Our group's product is safe to be implanted in mice and can emit light within certain wavelength requirement. The light source can also be switched on and off easily by operator for research use. The light's intensity is able to trigger the cells inside the lung of mice.

Client Requirements:

The goal of our client is to use optogenetic activation to alter immune cell functions in mice to understand inflammatory responses in brain and lung diseases. In vivo light delivery is key to this project and our client needs a solution for 470 nm and possibly 405 nm light that can deliver light to a larger area, which is about 1 cm in diagonal, and can be switched on and off for hours in the mice. The heat produced by the light should neither be harmful nor kill the cells and tissues near implantation site. The light should be delivered deep enough to stimulate the lung of the mice. The light should also be reusable if it is expensive to develop.

Design Requirements:

1. Physical and Operational Characteristics

a. Performance requirements:

Light must have a size of approximately 1 cm with a broad light source range able to penetrate deep into the organs of the mice. It also needs to have a wavelength of 405 nm and/or 470 nm without producing UV rays.

Project 1 Additional requirements: Light source must be composed of a flexible biosynthetic material able to operate in conditions within the mices' bodies. It needs to have a wireless system so operator can switch between the 405 nm and 470 nm wavelengths.

Project 2 Additional requirements: Light source must have an automated way which will allow it to switch on and off for 30 second intervals over 24 hours. Must be flexible and able to stick on the outside of the mice's skull.

b. Safety:

The heat generated by light should be minimal and not be harmful for neighboring cells and tissues. The production of UV rays by the light source would also cause harm to the cells. The material should also be biocompatible so that it could not cause an inflammatory response.

c. Accuracy and Reliability:

The light needs to be durable and biocompatible so that it is able to withstand the environment inside the blood vessels of mice. Also, the light source developed should be broad enough to cover enough areas on the organs of the mice to make sure the light-sensitive genes can be triggered and monitored.

d. Life in Service:

Ideally the device will be disposable and only used once. If our product is expensive then it needs to be reusable. The light source should also work continuously and consistently without unpredicted damage in the hardware.

e. Operating Environment:

Under the rib cage and by the lungs of the mice.

f. Ergonomics:

Make design easy to handle, such as make grooves so tweezers or fingers can pinch it easier and the syringe can take it in and expel it readily.

g. Aesthetics, Appearance, and Finish:

The design needs to be small, compact, and streamlined. Since the design will be used in vivo, wires are acceptable but not preferred. The materials used need to be durable and able to function when in the blood vessels of the mice.

2. Production Characteristics

a. Target Product Cost:

The client did not specify the budget as long as we make reasonable use of the money provided by our client. Our team will try to minimize the amount we might spend and try to make our device reusable and reliable.

3. Miscellaneous

a. Standards and Specifications: FCC Regulation of Wireless Medical Devices

Our device to be built might use a certain type of wireless transmitter. According to Equipment Marketing and Authorization of FCC regulation, every type of wireless transmitters being used must be certified for compliance with the FCC's rules before it can be marketed in the U.S. The certification process involves Testing, Radiation Exposure, and Device Labeling.

b. Customer:

For a preliminary design specification in regard to customer, the device should be user-friendly (easy to handle, will not fall apart easily when mishandled, etc).

c. Patient-related concerns:

Our design will not be applied to patients directly even though the ultimate goal might be to alter immune response of human. For our research subjects, mice, the use of light source must not be detrimental to the research projects and the device should be safe to mice when being implanted in mice.

d. Competition:

1. Biocompatible optical fiber-based nerve cuff can be used for light delivery that wraps around the target neuron. The research mainly considers light delivery to peripheral axons [1].
2. Epidural fiber-optic implants:
   Epidural fiber is used in light delivery for spinal cords. The system [2] enables sufficient light intensity and different light wavelength to be delivered.

Reference:

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Appendix II: Matlab Code

```
file = '...';
A = load(file);
reference = [470.2540, ...
  470.6100];
wavelength = A(400:1400,1);
intensity = A(400:1400,2);
Light_Energy = zeros(size(wavelength,1),1);

for i = 1:size(wavelength,1)
    Light_Energy(i,:) = intensity(i,:) * 10 * 3e8 *6.63e-34*1e9/(wavelength(i,:));
end
p =
plot(wavelength,1000*Light_Energy*10000/(0.025^2*2048*14e-6*200e-6),'b-','LineWidth',2);
ylabel('Light Intensity (mW/cm^2)');
xlabel('wavelength(nm)');
```

Appendix III: Arduino Uno Code

```
#include <Adafruit_DotStar.h>
#include <SPI.h>
#define NUMPIXELS 144 // Number of LEDs in strip
#define DATAPIN    4
#define CLOCKPIN   3
Adafruit_DotStar strip = Adafruit_DotStar(NUMPIXELS, DATAPIN, CLOCKPIN);
unsigned long time;
unsigned long previous = 0;
unsigned long current;
void setup() {
```

```
#if defined(__AVR_ATtiny85__) && (F_CPU == 16000000L)
  clock_prescale_set(clock_div_1); // Enable 16 MHz on Trinket
#endif
  strip.begin(); // Initialize pins for output
  strip.show();  // Turn all LEDs off ASAP

}


void loop() {

    strip.setBrightness(80);

    strip.setPixelColor(0,255,169,0);
    strip.show();

    delay (30000);


    strip.setPixelColor(0,0);
    strip.show();
    delay (30000);

  }
```

Appendix IV: Materials Cost

Item	Description	Manufacturer	Part Number	Date	QTY	Unit Cost	Total	Link
Adafruit Trinket - Mini Microcontroller - 5V Logic	The microcontroller that can control the LED	Adafruit	1501	10/17/2018	3	$6.95	$20.85	https://www.adafruit.com/product/1501?gclid=EAIaIQobChMI46W6gtyO3gIVDy9pCh2c-QEFEAQYASABEgJzzPD_B

								wE
DotStar Micro LEDs (APA102–2020) - Smart SMD RGB LED - 10 pack	The 2mm*2mm tiny LED that can emit 465nm to 470nm light	Adafruit	3341	10/17/2018	2	$5.95	$11.90	https://www.adafruit.com/product/3341?gclid=EAIaIQobChMI7oPnz92O3gIVAwVpCh35EAELEAQYAyABEgJkVPD_BwE
M-D Building Products 12 X 24 Galvanized Steel Sheet 57321	12"x24" steel sheet	Ace Hardware	5686514	10/31/2018	1	$11.99	$11.99	https://www.acehardware.com/departments/hardware/metal-sheets-and-rods/steel-sheets/5686514?x429=true&utm_source=google&utm_medium=cpc&gclid=CjwKCAjwpeXeBRA6EiwAyoJPKm9-XYbLbjUTqwzUvVufRTzu4sR3MZJ2HGNPBpE_GA1g7-FkGfhA6BoCl6wQAvD_BwE
PDMS	Transparent Biomaterial	McClean LAb	N/A	11/29/2018	1	$0.00	$0.00	N/A
SMD Paste	THERMALLY STABLE SOLDER PASTE	Chip Quik Inc.	TS391LT	10/17/2018	1	$16.95	$16.95	https://www.digikey.com/product-detail/en/chip-quik-inc/TS391LT/TS391LT-ND/7802220?WT.srch=1&gclid=EAIaIQobChMI85u8xeuO3gIVkoppCh0wEQoIEAQYASABEgIxn_D_BwE
Circuit Board	Help with LED soldering	Team Lab	N/A	12/1/2018	3	$1.00	$3.00	N/A
Arduino	Microcontroller	Arduino	N/A	Last Semester	1	$0.00	$0.00	N/A

Uno								
						Total Cost:	$64.69	