Illuminating Cellular Regeneration: Optogenetic Engineering

Capstone Design Report
Wake Forest University Department of Engineering

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Team Member Names

By signing, students consent to have reviewed the contents of this report and to agree with the content presented.

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EXECUTIVE SUMMARY

Our team has presented an overview of the progression and key aspects of our capstone project, which focuses on designing and developing an optogenetic platform to support Dr. Josh Currie's research on axolotls. This research aims to determine the molecular, cellular, and self-organizational principles that separate regeneration from fibrotic wound healing. The Mexican Axolotl, *Ambystoma mexicanum,* is important to this research as the aquatic salamanders are known for their regenerative capabilities, offering unique insights into tissue repair and regeneration. To assist this research, our project aims to design a versatile and precise optogenetic device capable of controlling light exposure for specific wavelengths, radiance, and periods. This involves using light to control and manipulate cells that have been genetically modified to express light-sensitive proteins within the axolotls, allowing for the study of their regenerative pathways.

The significance of the project lies in its potential to advance the field of regenerative medicine. We aim to enable new insights into tissue repair and regeneration by supporting research on the mechanisms of axolotl regeneration using optogenetics, providing insights into fundamental biological processes with potential applications across species. This fosters interdisciplinary collaboration among researchers and students from various backgrounds, cultivating an understanding of regenerative processes and optogenetic innovation.

During the Discovery Design phase, our team analyzed the problem statement, investigated relevant background information through existing literature reviews, and consulted with stakeholders to define precise system requirements. This groundwork laid the foundation for a solution tailored to Dr. Currie's specific research needs, emphasizing innovation and functionality in optogenetic applications.

In the Conceptual Design phase, our team generated, evaluated, and refined various concepts, leading to the selection of a viable design strategy that optimizes light delivery for axolotl regeneration studies. Our efforts culminated in developing a proof-of-concept design that combined ease of use with precise control, setting the stage for the embodiment and detailed design phases.

During the Embodiment Design phase, our team refined the initial prototype using 3D modeling, cardboard mockups, and laser cutters, allowing us to work hands-on in developing the optogenetic system for Dr. Josh Currie's axolotl regeneration research.

In the Detailed Design phase, our team completed the final prototype (*The LumiGen Platform*) using high-quality acrylic, installed the necessary electrical components, and refined our programming to ensure the device could meet the precise requirements of optogenetic research. This final prototype is now fully operational and will be integrated into Dr. Currie's laboratory to support advanced studies in tissue regeneration, providing controlled light exposure essential for scientific investigation.

1 DISCOVERY DESIGN

1.1 Problem Statement

Axolotls are aquatic salamanders recognized for their ability to regenerate lost limbs, offering a unique window into understanding regenerative pathways distinct from typical wound healing observed in most mammals. With common fibrotic wound healing, tissues close up, often resulting in scar formation, whereas regeneration involves the rare capacity to regrow parts of organs or tissues after damage or amputation. Dr. Josh Currie's lab is leveraging optogenetics to delve into axolotl regenerative pathways to uncover the underlying mechanisms that enable this capability. However, the lab faces a technical challenge: they require a specialized "lightbox" to manipulate light-sensitive proteins through exposure to specific wavelengths of light to collect the data they need. By manipulating light-sensitive proteins through exposure to specific wavelengths of light, it is possible to turn on/off gene transcription or to recombine DNA. The "lightbox" must illuminate the specimen's targeted areas for defined wavelengths, radiance, and durations. Our team possesses the technical experience needed to develop a functional prototype, aiming to advance the state of optogenetics research at Wake Forest University.

1.2 Mission Statement and Broader Impact

The RegENgineers are driven by a profound commitment to advancing scientific knowledge and regenerative medicine. We aim to design and construct an innovative optogenetic lightbox dedicated to studying Mexican Axolotls and their regenerative capabilities. Further research on these creatures can inspire breakthroughs in tissue regeneration, and we envision our lightbox can benefit society in several ways:

1. Delving into the mechanisms of axolotl regeneration using optogenetics, there is a potential to provide new insights into tissue repair and regeneration, ultimately leading to improved treatments for traumatic injuries, degenerative diseases, and age-related conditions.
2. Understanding axolotl regeneration can lead to the development of more effective wound healing and tissue repair treatments. This could reduce the need for extensive surgeries, organ transplants, and prosthetics, ultimately improving the quality of life for individuals with injuries and medical conditions.
3. A working optogenetic lightbox would foster interdisciplinary teamwork of researchers and students from diverse backgrounds, including biology, engineering, optical physics, and genetics, cultivating a holistic understanding of regenerative processes and optogenetic innovation.

1.3 Methods and Analysis for Generating System Requirements

1.3.1 Stakeholder Analysis

This project is designed to assist The Currie's Lab's specific research goals on axolotl regeneration. As the primary investigator leading the lab, Dr. Currie's specific requirements have informed our project scope and defined research goals. Hence, maintaining constant communication with him throughout the development of our prototype is a priority to ensure we are meeting his needs. Other stakeholders include researchers, scientists, technicians, and graduate or undergraduate students who could be involved in the laboratory's day-to-day activities. Researchers and scientists within the broader scientific community can be considered stakeholders who share their findings and contribute to the collective knowledge of optogenetics. In addition, other optogenetics labs can modify our product to meet their specific needs once it has been developed. The RegENgineers intend to design the equipment to be both accessible and adaptable to emerging trends in optogenetics research, which can impact biomedical research and allow researchers to gain a more comprehensive understanding of the field.

Regulatory bodies that oversee and evaluate the ethical and humane treatment of animals in research are also important to consider. Regulatory requirements may evolve, and uncertainties could arise regarding interpretation or changes in standards. Public attitudes towards animal research may change, and uncertainties in public perception will be considered when designing the equipment.

1.3.2 Background Research: Literature Review

The Mexican Axolotl, *Ambystoma mexicanum*:
The unique physiological characteristics of axolotls necessitate specialized considerations to ensure their well-being in captivity. Literature reveals that appropriate temperature and humidity maintenance are crucial to axolotl health. Axolotls require low light settings and colder water since they originated in higher altitudes at 16-24°C [1]. Axolotls are nocturnal creatures that are very sensitive and vulnerable to intense light, especially UV rays. This information led us to conclude that it is vital that we do not stress the creatures by way of overheating due to overexposure to intense light.

Providing a suitable environment in the lightbox is a key aspect of their care. According to the American Society of Ichthyologists and Herpetologists guidelines for the use of live amphibians and reptiles in field and laboratory research, the frequency of cage cleaning should represent a compromise between the level of cleanliness necessary to prevent disease and the amount of stress imposed by frequent handling and exposure to unfamiliar surroundings and bedding [2]. This information informs our project as it necessitates the ability to control and clean conditions. Additionally, the literature underscores the significance of proper handling techniques, emphasizing the need for gentleness and minimizing stressors to prevent potential harm.

LED Requirements:

Upon review of the appropriate illumination delivery system when selecting a light source for optogenetics, it has been determined that selecting a light source closer to the peak target wavelengths and sufficient intensity is most suitable to achieve optimal activation. The required light intensity for an experiment depends on the field of view and the opsin (the universal photoreceptor molecules of all visual systems) the researcher selects [3]. The objective will determine how much area is required to be covered by the light source.

From speaking with Dr. Currie, the target wavelengths for the emitted should be 460nm (Blue) and 660nm (Red). A thorough examination of existing literature reveals a growing emphasis on optimizing illuminance, the amount of light that shines on the surface (lumens/m2), and irradiance, the amount of optical power or radiant energy striking a given area of a surface (mW/cm^2), both parameters for successful optogenetic manipulation in axolotls [4]. Our light intensity research shows that the activation energy required for the main protein of focus (ChR2) is less than 1 mW/cm^2 [5]. Studies also underscore the importance of selecting LEDs with specific wavelengths that align with the absorption spectra of the light-sensitive opsins employed in axolotl optogenetics.

Regarding illumination delivery, focus on light spread and scattering from the source is pivotal. The further a specimen is from the end of the source, the more power is needed at the source in order to have sufficient irradiance to activate the opsin expressed in neurons, which can be seen in Figures 1. and 2. [6]. Based on Figure 2 and LED testing results in Appendix 2, we would define our threshold irradiance at 1.5 inches for 12 volts - the activation distance furthest from the source of illuminance. Additionally, researchers highlight the significance of achieving precise control over illuminance levels and pulsing to elicit targeted responses, specifically on axolotl limbs [7]. The stress on the quantification of irradiance and the need for uniform light distribution within the experimental space ensures consistent and reliable outcomes.

Our team plans to consider these parameters when selecting the proper LEDs for the lightbox. In the future, we will compare LED models and their performance in meeting the desired system requirements to elicit responses in axolotls, providing valuable insights for seeking to refine the optogenetic lightbox. The selection of appropriate LEDs for optogenetic research in axolotls is a critical aspect influencing the precision and efficacy of experiments. LEDs are low-cost and eye-safe, having a long lifetime, making them an excellent choice for any lab looking to perform optogenetics [3]. They come in a wide range of wavelengths (UV to NIR); thus, the appropriate LED can be found to best fit a researcher's choice.

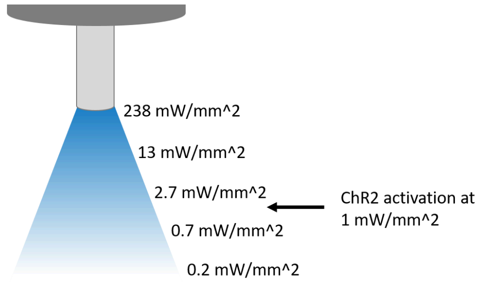

Figure 1. *Change in irradiance over distance* [6]

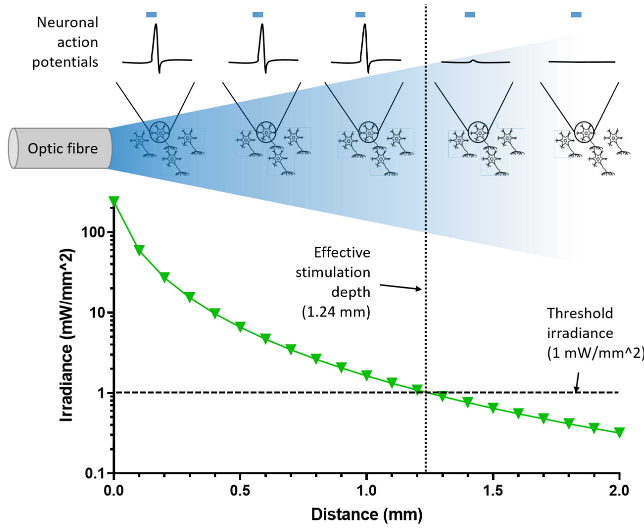

Figure 2. *Threshold irradiance [6]*

1.3.3 Benchmarking of Existing Solutions

A few of the current optogenetic solutions include the LITOS tool [8], the LAVA board [9], and the lightbox design created by Justin D Vrana [10]. While these particular light boxes are seemingly used for smaller specimens, they each address some aspect of our problem, particularly for light configuration. The LITOS tool is inexpensive to assemble and has a high spatiotemporal resolution due to the reliance on an LED matrix display. While a solution like this may not fully isolate a limb, it does emphasize illuminating regions programmed into the display, which can be used to illuminate multiple cell culture dishes simultaneously. The lightbox Justin Vrana developed has characteristics such as pulse length, intensity, and interval, which can repeatedly activate target proteins. The LAVA board has a more complex build than the other two designs, as it provides greater control over the spatiotemporal settings and light output. This solution also considers temperature, as it includes a built-in heat sink. The heat sink on the LAVA device will take in the heat generated by the circuit and dissipate it away from the device. This characteristic makes it a suitable benchmark for our prototype, which may need a source of temperature regulation to avoid overheating the specimen and other surrounding components. Our prototype will require some form of spatial customizability, various light configurations, and temperature control. Its development must be within our budget of $2,000. Our device will differ from these existing solutions because its spatial and light configurations will be specific to axolotls of a certain age range.

While the LAVA board meets all of the broader requirements for our device, as shown in Table 1, its spatial patterns are made specifically for use with cell cultures. We can learn from these solutions that there are multiple ways to configure the light output that can be customized for our needs. The LAVA board presents the most significant aspects we desire for the prototype. However, note that the LAVA board itself would not be sufficient for this project because it lacks several other unique features that our stakeholders would need to be specific to axolotls within a certain age range. Overall, the spatial configuration needed to target specific limbs of the specimen will require the most consideration. Initially, Dr. Currie did this manually by sedating the axolotls before covering most of the petri dish using black tape. He would use a single LED light strip to focus on one of the limbs. The results of this would be variable and would not always guarantee the animal's survival. None of the options listed below are commercially available and must be assembled following the instructions provided by each creator.

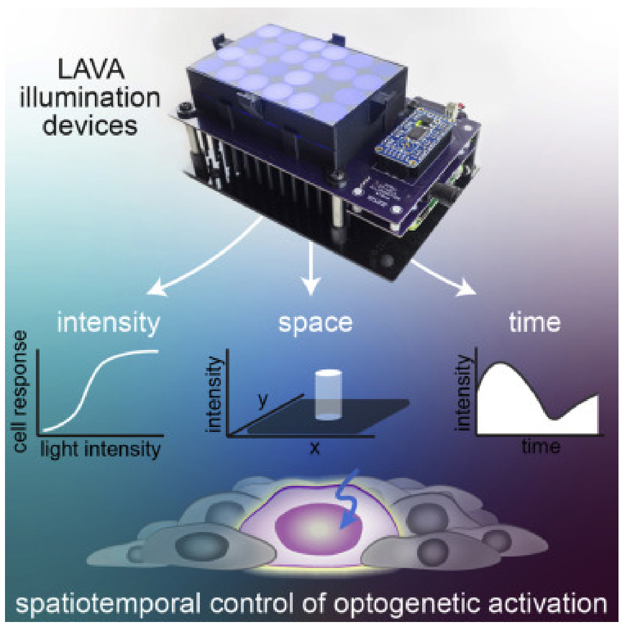

Figure 3A. *The LAVA device [9]*

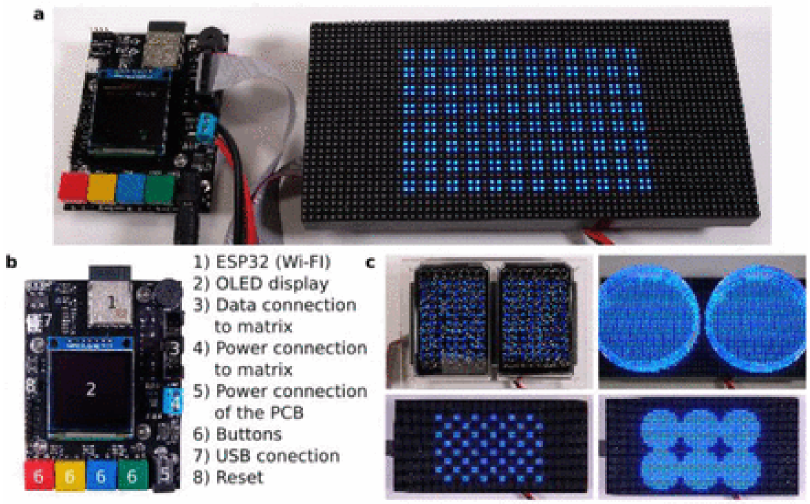

Figure 3B. *The LITOS device [8]*

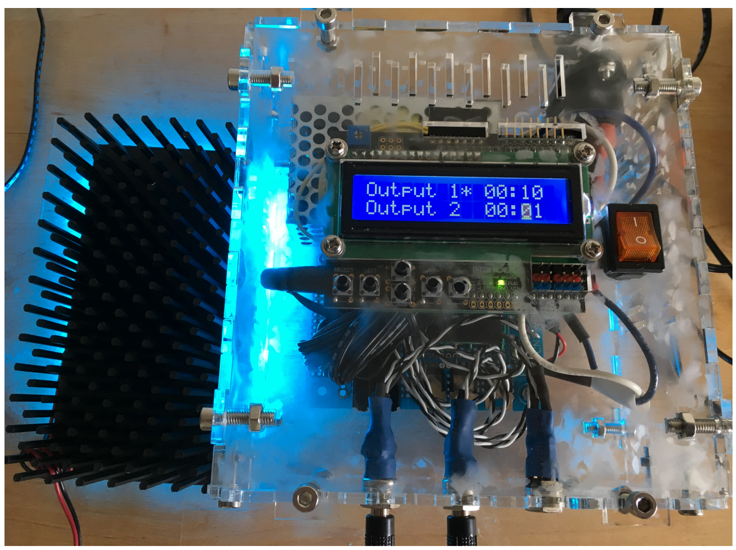

Figure 3C. *J.Vrana's device [10]*

Table 1: Comparison of benchmark solutions.

Device	Light configuration settings (intensity, wavelength, etc.)	Temperature Control	Spatial configuration setting	Can be used for a larger specimen (axolotl)
LITOS	☑	☐	☑	☐
J.Vrana's device	☑	☐	☐	☐
LAVA	☑	☑	☑	☐

1.3.4 Standards, Codes, Regulations

Since our project involves working with live axolotl specimens, our relevant standards center around animal handling protocols. Listed below are the standards we consider when working with live specimens as well as general research considerations:

Institutional Animal Care and Use Committee (IACUC)

- Ensure that our project complies rigorously with the ethical standards set forth by the IACUC. This ensures the well-being and ethical treatment of the axolotl specimens throughout our research.
- The U.S. Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals regulations serve as the foundation of our ethical responsibilities in conducting research with live animals.

Animal Welfare Regulations

- We have identified guidelines for amphibian research here and will refer to and reference these guidelines in greater detail as we move into testing our prototype. More work is needed to identify those issues particularly relevant to our project.
- Dr. Currie has previously outlined specific regulations due to the nature of his research, and thus, we will continue to abide by these appropriate protocols under his supervision as we begin testing.

Commitment to these codes, standards, and regulations reflect our dedication to the animals' welfare in our project. Appropriate animal handling procedures should be followed by all researchers operating our device.

1.3.5 Preliminary Testing for Problem Scoping

Understanding the design problem and parameters involved meeting with Dr. Currie, our primary stakeholder, to determine the scope of the problem and his specific lab needs. We examined an initial prototype he had designed this summer to understand his objectives and to establish a foundation for our conceptual design. Dr. Currie's prototype consisted of a crude model of a petri dish covered with black tape on a portion of the bottom, allowing an LED strip to illuminate specific limbs (Figure 4). In this prototype, the black tape partitioned off areas Dr. Currie wanted to expose to light while enabling limb illumination by the LED. However, temperatures in this initial prototype were too high for axolotl vitality to be maintained, and there was no means of specific control over timing or allowance for testing alternate wavelengths. This review of the prototype was crucial to our concept design phase and provided us with a clearer understanding of Dr. Currie's vision for the project's deliverables. Maintaining constant communication with Dr. Currie and periodically checking in as we continue our design process is crucial to ensuring our product meets his lab needs.

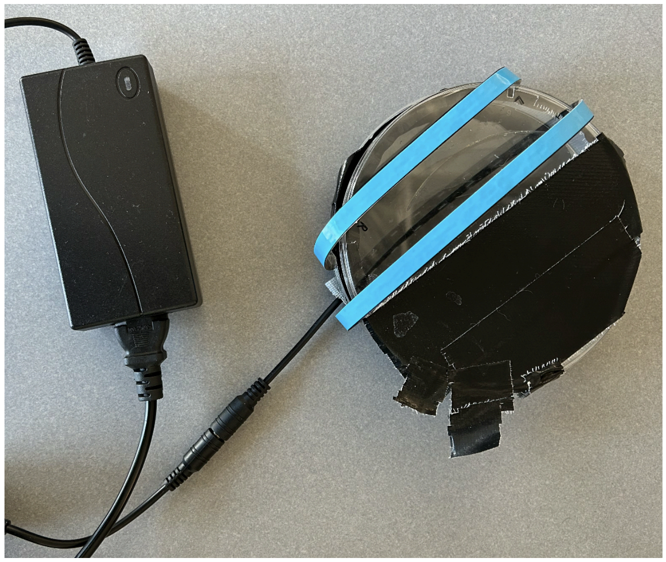

Figure 4. *Dr. Currie's original prototype*

While we will maintain Dr. Currie's initial design focus on limb partitioning and illumination, we seek to improve it by emphasizing a safe means of illumination with a higher degree of control. See Section 1.4 for the specific system requirements we identified based on these initial goals.

1.3.6 Engineering Calculations for Deriving System Requirements

Defining our preliminary system requirements required communicating with Dr. Currie to determine appropriate standards prioritizing axolotl specimens' health and safety. These requirements are outlined in greater detail in Section 1.4. As we transition into the Conceptual Design phase of this project and begin to think in greater detail about our prototype and which parts we will be implementing, we will be able to complete calculations to determine appropriate power/wavelength outputs based on our system components as well as any other calculations we deem appropriate. Calculations for light coverage are documented in Section 2.3.

1.4 System Requirements Prioritized and Justified

To begin outlining our system requirements, we collaborated with our stakeholders to understand their specific needs and expectations. Stakeholder requirements encompass three main aspects: affordability, accessibility, and component specificity. Affordability guides our decisions regarding component selection for use in creating the prototype, considering that many of the engineered solutions in this field can be very project-specific and are not considered an appropriate option for working on optogenetic research projects. Accessibility emphasizes the importance of the device being operated by non-engineers or individuals with minimal technical training, including Dr. Currie. Finally, component specificity is concerned with delivering specific wavelengths of light that can illuminate target areas of the organism while maintaining optimal living conditions for the axolotls.

System requirements were developed in greater detail from these general stakeholder requirements (see Table 2 for specific breakdowns of requirements). We determined quantifiable power/wavelength outputs, temperature control, light filtering, and distinct wavelength settings to be most important when considering component specificity (see Section 1.3.2 for related literature). Regarding accessibility, allowing for adjustable containers and simple setting changes allows Dr. Currie and other researchers to operate the device independently. To maintain affordability, we remained cognizant of our budget restriction and attempted to keep costs as low as possible. Though maintaining regulatory compliance and implementing safety measures were not outlined in our stakeholder requirements, our team wanted to acknowledge these as we further develop our design.

Table 2. System Requirements. Identified system requirements with quantified ranges.

Requirement	Description
1. Quantifiable Power	The device must provide a power output of 1 mW/mm^2 with an accuracy of 0.01 Watts
2. Wavelength Outputs	The device must provide a quantifiable wavelength output of 460 and 660 nm with an accuracy of ± 25 nm.
3. Temperature Control	The device must maintain an internal temperature between 18°C and 22°C to ensure the axolotls' comfort and well-being.
4. Adjustable-Size Containers	The device should have adjustable-size specimen containers capable of accommodating axolotls ranging from 5 cm to 20 cm in length.
5. Light Filtering	The specimen containers must filter 95% of the directed light to protect the rest of the axolotls' bodies while exposing only a single limb.
6. Distinct Wavelength Settings	The device should offer two or more distinct wavelength settings to allow for versatile experiments.
7. Safety Measures	The device must incorporate safety features to prevent harm to both the axolotls and users.
8. Regulatory Compliance	The device must comply with relevant safety and ethical regulations for the use of axolotls in experiments.
9. Budget Consideration	The cost of device components must fall below $2,000.

2 CONCEPTUAL DESIGN AND ANALYSIS

2.1 Concept Generation

In the initial stages of concept generation for our optogenetic system, we employed a combination of brainstorming sessions and morphological matrices during collaborative discussions within the team. Furthermore, we incorporated direct design principles, guided by user-centered design considerations, following insightful consultations with our stakeholder, Dr. Currie. The objective was to produce a diverse array of conceptual solutions that could effectively address the system requirements and cater to user recommendations we had identified.

The artifacts stemming from this phase include detailed sketches that encapsulate the essence of our primary concept, colloquially known as the "Air Fryer" design. These visual representations specifically showcase key components, such as the removable tray featuring Petri indents for experimental setups, various powering methodologies, and distinct illumination design options. Each team member generated their own concept from this “Air Fryer” model (Figure 5). Each alternative within the "Air Fryer" model possesses distinguishing features carefully crafted to align with our system requirements, drawing inspiration from comprehensive background research and benchmarking efforts.

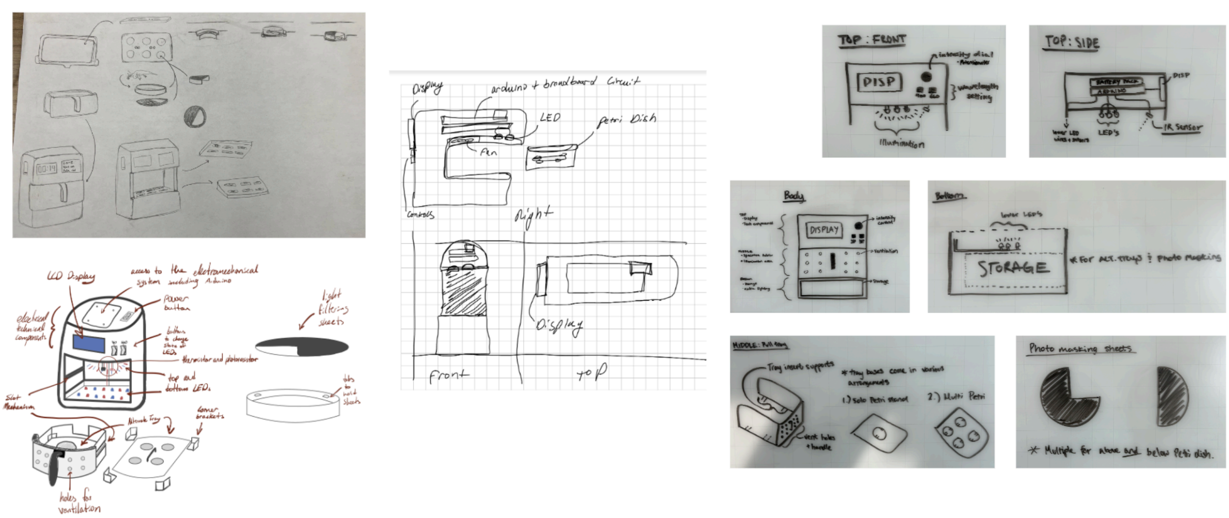

Figure 5. *Design breakdown from individual team members.*

Team members met and discussed the key distinctions between each design, which were then displayed in a morphological matrix (Table 3). From here, team members systematically narrowed down these ideas to our final concept (see Section 2.2 for detail on evaluation processes).

Table 3. *Morphological matrix representing individual concepts and key distinctions between concepts.*

Design Block	Elena's design	Kyle's design	John's design	Sarah's design
Photomasking	Top cap, bottom sheet	Petri Specific Covers	Petri has tabs to hold photo masking sheets about specimen	Removable attachment
LED illumination	Array of 12 LEDs top and bottom, 460/660 nm next to each other	Led array with and even distribution of 460/660 nm lights.	Array of LEDs of each wavelength evenly distributed; 20 LEDs on the top and bottom and half 460nm/half 660 nm	Individual LEDs/matrix board fixed to top of device beneath rest of circuit
Tray design	Non removable, set 6 containers	Removable tray with adjustable base to allow for ease of access	Tray with engraved holes to hold the petri dishes, handles on the sides to lift tray out of basket	Fixed tray, built into main body
Display	Separate timer display and temperature/ wavelength setting/pulse mode display	LCD display that displays outputs and current status (time, temp, wavelength)	LCD that displays ambient temperature, and time elapsed during experiment located on top above the basket	LCD that displays the power output, and selected LED wavelength.
Petri dish containment	"Lipped" circular divots	Fitted Spaces within tray	Regular 3D printed petri dishes with tabs in the inner walls to hold the thin filter sheets when placed on top of the dish	Placed on top of fixed tray
Temperature sensing	Ambient sensor	IR and ambient sensors	Thermistor ambient sensor	IR and ambient sensors
Device power	Wall connection	Wall connection	Wall connection	n/a

The "Air Fryer" concept's notable feature is its emphasis on simplicity, ensuring ease of use for individuals operating the optogenetic system within a laboratory setting. This deliberate design choice was made to facilitate intuitive understanding, allowing users with varying levels of expertise to operate the device seamlessly. Considerations such as power requirements and optimal illumination durations were pivotal in influencing our design decisions, underscoring the importance of meeting specific criteria outlined in the system requirements.

2.2 Concept Evaluation

As previously discussed in this report, we identified key distinctions between our individual air fryer designs (Table 3). Group discussion and Pugh chart generation enabled us to systematically evaluate each of our ideas to come to a final consensus. These decision-making processes are outlined below.

<u>Photomasking</u>

Photomasking allows us to isolate specific limbs of the axolotl by blocking light. We discussed implementing plastic petri disc "caps", sheet cutouts, or a silicon cover directly over the axolotl as a means of preventing unnecessary light exposure. Evaluating under the criteria of coverage, longevity, cost, and adaptability (Table 4), we determined 3D-printed plastic petri dish caps to be the superior design choice. They provided the most coverage and longevity while maintaining a relatively low cost. Though not as adaptable, we came to the conclusion as a team that this would not be a major concern, because the materials are easily accessible and it would be easy to either adjust and reprint if needed or to print duplicates.

Table 4. *Weighted design matrix for photo masking sheet design*

Criteria	Weight	Plastic Petri Dish Caps	Sheet Cut-outs	Silicon Cover
Coverage	0.5	4.5	3	2.5
Longevity	0.1	5	2	4
Cost	0.1	4	5	4
Adaptability	0.3	2	5	3
Total Score	1	3.87	3.75	3.37

LED Illumination

From our initial discussion, we planned to implement an LED array with an even distribution of 460/660 nm diodes on both the top and bottom of the device. We were in consensus about using individual LEDs for the design and making this choice only involved discussing the arrangement of an array of lights that could be alternately lit based on the desired wavelength. However, once we progressed into the prototyping phase of the design and began to look for specific LEDs, we found our initial options were not as wavelength-specific as we had hoped and learned we would have to consider alternative means of illumination. See Section 4.4 for greater detail on how we addressed this issue for our final design.

Tray Design

Tray design was another point in which team members were in near agreement. Basic design configuration includes 4 individual wells in which petri dishes can be placed to allow for bidirectional illumination with a lipped edge to secure them. Pegs were also proposed as an option, but we ultimately decided they may be prone to breakage or would be more difficult to implement. We decided to implement 4 wells based on discussion with Dr. Currie about how many experiments he hoped to be able to perform at once, and we believe this number will allow us to ensure even illumination across specimens. The tray will be easily removable for cleaning purposes and to allow for access to internal components of the device if there is a need for maintenance.

Display

Our display will present ambient temperature and time elapsed during the experiment. Our design will also be programmable for different periods of time based on the needs of the researcher. With an I2C LCD, only a few lines of code are needed to achieve complex graphics and text display features, meaning it presents as a likely option. However, at this phase of the design process, we are more concerned with display configuration and plan to discuss the most appropriate electronic components further in the embodiment design process.

Petri Dish Containment

As discussed in the “Tray design”, we selected lipped circular divots to contain petri dishes within the device. Dr. Currie provided the petri dishes he hoped to implement himself so that we could determine the appropriate dimensions for our device, meaning he ultimately was more responsible for this design component.

Temperature Sensing

We explored a few different options for non-contact sensing to preserve axolotl safety throughout experimentation. Ultimately, our decision came down to the MLX90614 IR sensor versus the DHT 22 Thermistor (Table 5). We considered ease of implementation, accuracy/precision, small size, low price, and simplicity of data interpretation in narrowing down to our final choice. By these measures, the DHT22 thermistor emerged as the preferred choice. Not only did it outperform in size and price measures, but we found that the values it would give us were easier to interpret than those by the IR sensor. Ease of implementation (connecting to a circuit) and accuracy/precision (within 5°C) were the same for both sensors. The IR sensor provides surface temperature while the thermistor measures ambient temperature. In order to determine surface temperature, the IR sensor requires a series of complicated calculations while the thermistor output is a single measure for ambient temperature. We have a number of sources for appropriate ambient temperatures for axolotls, but none for what the temperature of their skin should be. The thermistor also has humidity capabilities, which we can factor in later if this is something we find we should consider in our design.

Table 5. *Weighted design matrix for temperature sensing design*

Criteria	Weight	IR Sensor (MLX90614)	Thermistor (DHT 22)
Ease of implementation	0.1	5	5
Accuracy/precision	0.3	5	5
Small size	0.15	3	4
Low price	0.15	4	5
Simplicity of data interpretation	0.3	2	5
Total Score	1	3.65	4.85

Device Power

We had several options to consider when it came to powering our device but ultimately the wall cable emerged as the best choice (Table 6). We considered reliability and durability, ease of implementation, cost, safety, power output, and consistency. Though it was outperformed on cost, it received high point allocations for the rest of the design considerations. Initially, we implemented the weighted design matrix to consider the other designs as we worried about shorting. Once we realized this would not be a concern with the wall connection it became the easy choice for our design.

Table 6. *Weighted design matrix for device power design*

Criteria	Weight	Wall cable	External battery pack	Internal battery pack	Computer Connection
Reliability and Durability	0.4	5	4	4	4
Ease of Implementation	0.2	5	4	3	3
Cost	0.1	4	4	5	5
Safety	0.1	5	4	3.5	5
Power Output and Consistency	0.2	5	4	4	5
Total Score	1	4.9	4	3.85	4.2

2.3 Engineering Calculations and Analysis for Conceptual Design

<u>LED Radiance and Heating Considerations</u>
Given the potential for heat generation by the LEDs, we explored calculations for estimating the heat flux ($Q=m \times Cp \times \Delta T$) to predict thermal effects on the axolotls. However, the absence of a significant temperature increase during light testing validates the current design's safety and efficiency without further heat management modifications.

To directly monitor the effects of heating caused by the LEDs, we have incorporated a DHT22 sensor onto the tray. This addition allows real-time temperature monitoring within the device's environment. We have integrated a safety feature to respond to any temperature exceeding the recommended range for axolotls, which is up to 22°C. This feature will automatically shut off the device when it detects that the temperature threshold has been surpassed, ensuring the well-being of the axolotls by preventing overheating. This proactive approach enhances the device's safety and aligns with our commitment to creating a research tool that is both effective and considerate of the biological specimens' needs.

<u>Illuminance Calculations for Light Exposure</u>
The illumination provided by the LED arrays is critical for activating opsin proteins in the axolotls. Considering that illuminance is additive, the initial test values for LED illuminance can be effectively doubled, accounting for the presence of red and blue light sources. This adjustment is vital for meeting the revised system requirements for illuminance, now set at one mW/cm^2, accommodating a broader range of experimental needs.

Specifically, at 12 volts and 0.5 inches from the diffuser sheet, the illuminance for blue light is measured at 1.904 mW/cm^2, and for red light, it is 1.436 mW/cm^2. These values confirm the device meets the necessary illuminance requirements for optogenetic activation within the specified range. This conclusion was supported by direct measurements and the understanding that the combined effect of red and blue LEDs would adequately stimulate the target proteins without exceeding thermal thresholds.

This section's calculations demonstrate the careful consideration given to ensuring the optogenetic device's efficiency and safety regarding cooling, heating, and illumination requirements. The data supports the current design's adequacy for research purposes while highlighting the potential for future refinements based on ongoing experimental feedback.

Once we began testing, we found that LED placement was constrained more by box dimensions rather than power requirements. It was found that each strip was ⅜ in tall and divided into cuttable segments 2 in long. To make a perfect square of LEDs, it was determined that 12x12 would be appropriate dimensions. However, due to the physical constraints of the box materials, a much smaller space was available to work with. So, 26 10” strips of alternating red and blue LEDs were used in the prototype design. This produced an LED array of 10” by 9.75”, allowing appropriate space for wired connections. See the Detailed Design section for more dimensioning details.

Calculating the Quantity of Acrylic

Before making the beta prototype, one of the main purposes of the alpha prototype was to quantify the amount of materials we needed for the body before purchasing the acrylic material for the final design. To calculate the amount of acrylic needed to purchase, we first used the 3D designs from Fusion 360 to calculate the surface area of all the exterior faces of the body and the drawers. We only care about the area on a single side of every piece, not the area on the other side of the edges. Additionally, everything was made of ¼” sheets, thus the calculation becomes straightforward since we are only concerned about one specific material of a specific size. Figure 6. is a screenshot of Fusion 360 showing a wall of the light box being highlighted in blue with the surface area calculated, located on the bottom right.

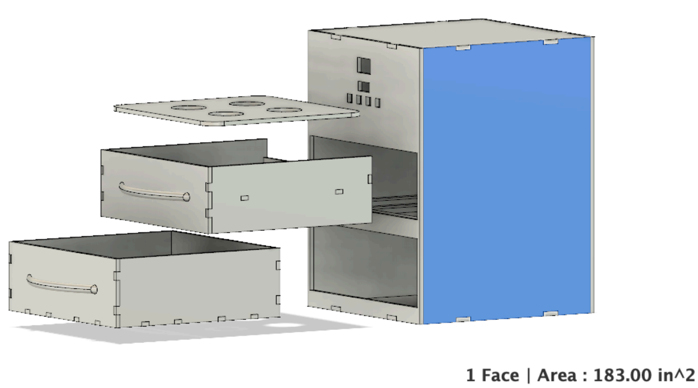

Figure 6. *A face of the light box highlighted in blue with the surface area of the selected face indicated below.*

The total sum of the surface area was calculated to be about 1540.2 in^2. The dimensions of the acrylic sheets that were purchased are 12x24 inches. The number of sheets required is the total surface area divided by 288 in^2, which was calculated to be about 5.35 sheets. The last step was to round up, which gave us a total of six 12x24 sheets.

The cost of ¼" opaque white acrylic sheets in size 12x24 inches from Makerstock is $17.95 per sheet. The total cost will run at about $107.70 to build one light box in terms of material for the body and excluding the electrical components. However, for the rapid prototyping phase, double the amount of acrylic needed was purchased in case of unforeseen mistakes in the laser cutting or assembly.

2.4 Concept Selection

As discussed in Section 2.1, our team iterated on our initial concept generation to generate individual air-fryer designs, which we then systematically evaluated as outlined in Section 2.2. Figure 7 shows the earlier design we generated in CAD to model the tray component of our final design. The next phase of our design will involve prototyping from this model and building upon it to meet the outlined system requirements. See the Detailed Design section for further component details.

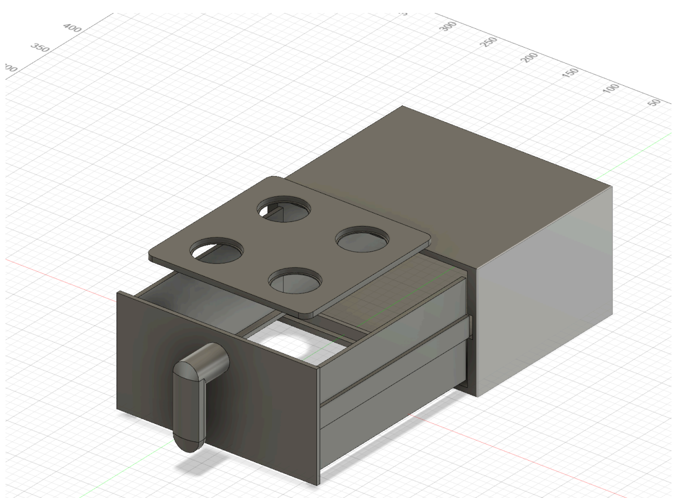

Figure 7. *3D concept generation of our tray*

2.5 Proof of Concept Prototyping

In assessing the feasibility of our conceptual solutions, we conducted proof-of-concept prototyping. We used a laser cutter to create a cardboard tray prototype (Figure 8), allowing us to evaluate its practicality and dimensions. Concurrently, we developed 3D digital prototypes for various components (Figure 9), aiding in the assessment of structural integrity and spatial relationships. Additionally, we employed 3D printing to produce prototypes of petri dish covers (Figure 10), evaluating their fit and durability. We also began to model our electrical components in Tinkercad (Figure 11) and have a working model of our timer. These prototypes helped in guiding decisions and in setting the foundation for a robust embodiment design phase.

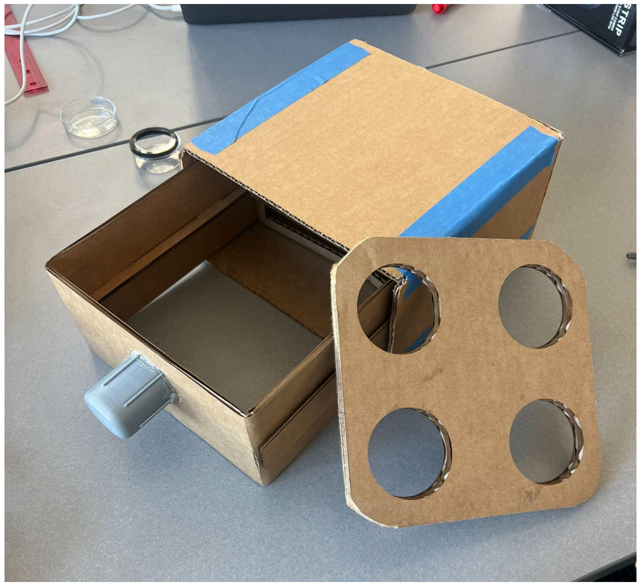

Figure 8. *Cardboard Tray Protype*

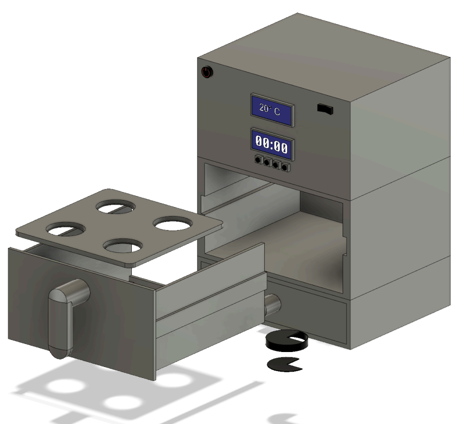

Figure 9. *Image of 3D Design Files*

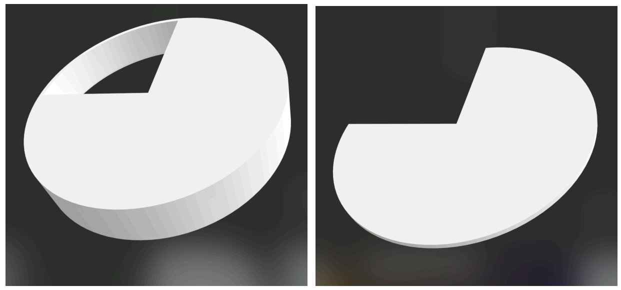

Figure 10. *Petri Dish Cover and Sheet*

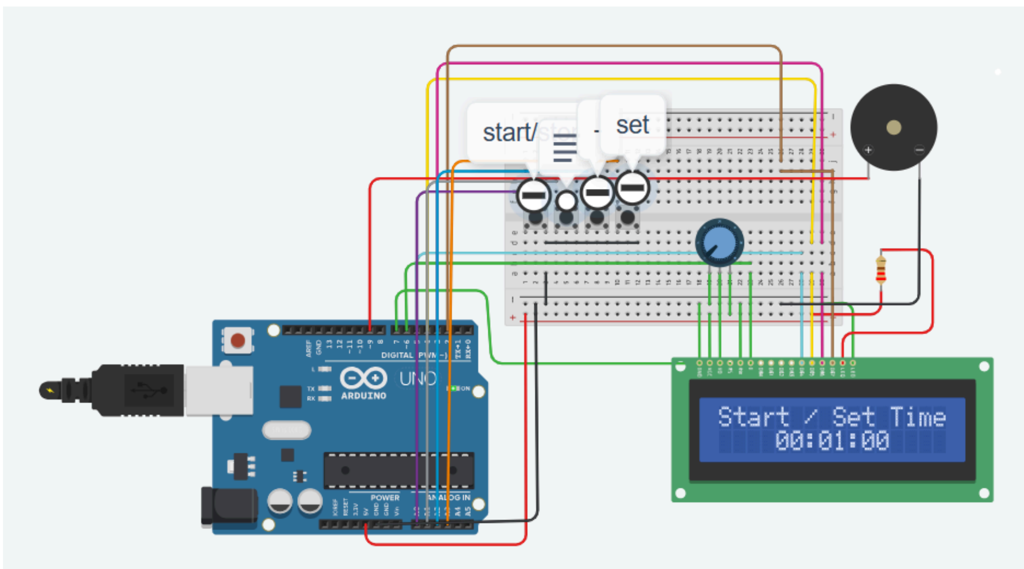

Figure 11. *Tinkercad model of a timer*

3 EMBODIMENT DESIGN

3.1 Preliminary Configuration and Architecture

Based on the chosen concept, the device is divided into two major systems: the basket and the electrical components. The basket consists of the containment and the tray where the axolotl specimens will be placed securely, along with the petri dishes, caps, and photo masking sheets. The electrical components consist of the subsystems of the temperature and timer displays and the LED array. In Figure 9, you can distinctly view the basket component separated from the main body and the displays in the top half of the body where the electrical components will go inside.

The basket will have slots so that when you put it back in the body, which has the bars on the sides, the basket will be secure and stable. The basket will contain a place to insert the tray in via a handle on the tray. The tray itself will have holes big enough for the petri dishes, which will have small ledges under the bottom of the wells to hold the dishes. The tray will be secure within the walls of the basket, that way it will not shift. This is supposed to work similarly to putting a shelf on the tabs when building a bookcase. To block light from illuminating the entire axolotl and shine on the limbs, there will be petri dish covers that cover above the axolotl, and there will be sheets placed under the petri dishes in the wells, to mask light coming from the bottom as seen in Figure 12. These components will be 3D printed.

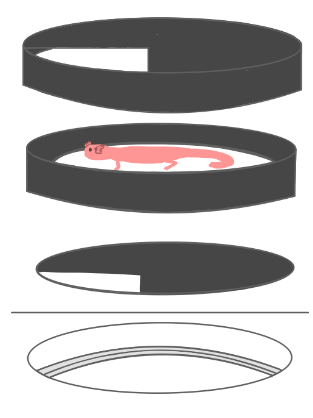

Figure 12. *Assembly of the dish cover, the dish itself, and the sheet in the well*

So far, the basket component has been prototyped out of cardboard as seen in Figure 8. This mock prototype has succeeded in holding the tray and the petri dishes, as well as working like a drawer, which is exactly how an air fryer would mechanically work.

As for the electrical components, the idea is to place the Arduino, the wiring, and the displays within the top section of the body, while the LEDs and temperature sensors are extended to go in the middle section. To reiterate, the DHT22 sensors will be placed inside the body of the lightbox. The back wall of the basket will have an opening for the sensors so that when the basket is inserted it will not cover the sensors. The timer display will be a separate subsystem and this will simply display the duration of the experiment. Although we have yet to confirm a specific model of LEDs, the idea is for the LEDs to be placed in the ceiling and bottom inside of the body to ensure full illumination inside the basket.

We have successfully simulated the display timer using an I2C LCD on TinkerCAD as seen in Figure 11. Next semester we plan to digitally simulate an I2C 4-digit clock display. The DHT22 sensors are fairly simple to assemble using the Arduino so when we have successfully simulated models of the clock and temperature displays using an online circuit simulator & schematic editor, we will be able to build the systems, hopefully using one Arduino.

As for the LEDs, we plan to purchase a variety of models to test them using our cardboard prototype. More than likely, the LEDs will have to be powered using an external source. The goal is to successfully switch the color output with just a toggle switch, while the Arduino powers the displays and sensors. Next semester we will have a circuit model of the LEDs and we build further upon the cardboard. By March, we plan to have an alpha prototype that will incorporate all of the systems together and with the proper materials. The materials we will be using are undecided but the requirement is for the containment to be reflective and not absorb the light so that the intensity of the light increases.

3.1.1 Engineering Analysis and Mathematical Modeling for the Preliminary Design

Our first goal for the next semester is to identify a software tool that we can use to model the electrical components of our system. Having an extensive model will assist in judging the feasibility of our design before we begin testing and gathering experimental data. To date, we have been using Tinkercad for simulating the timer we plan to incorporate into the rest of the circuit. However, Tinkercad does not have all of the components we plan to use to model our overall electrical system. Ultimately, the software will need to be able to simulate each of the main functions and display their outputs. This includes the timer, display, LED setup, inputs, humidity, and temperature sensors. It would be able to provide a reference point for the values we could expect to get from testing each iteration of the circuit. We will primarily be focusing on the light output, as specified by our stakeholders.

For our testing, we will quantify light using the following equation:

$$P = I(z)\pi R(z)^2 + I_0 \pi R_0^2$$

In the equation above, P is the radiative power, I is the irradiance at a distance of z, and R is the radius of the cone of light created by the source [4]. This equation, which takes the distance of the light source from the subject into account, will allow us to evaluate the efficiency of our circuit through each iteration of the prototype. See section 3.3 for illumination measurements.

3.1.2 Environmental Design Considerations

In developing our optogenetic device prototype, we strongly emphasized environmental considerations, guiding our choice of materials and components towards sustainability and minimal ecological impact. We selected cardboard for our initial prototype, which was valued for its biodegradability and compatibility with recycled materials. Our design philosophy extends to the final design phase, where robust materials able to last through a number of uses (ex. PMMA acrylic) will be used to promote environmental stewardship further.

To reduce the device's energy footprint, we incorporated energy-efficient electronics, aligning with our goal to minimize energy consumption. A significant feature of our design is the ease of component replacement, which prolongs the device's lifespan and supports environmentally friendly disposal practices. This approach ensures that electronic parts can be recycled, mitigating the generation of electronic waste.

Manufacturing efficiency was another critical consideration. We have optimized the use of materials to cut down waste and plan for the recycling or repurposing of any leftovers. As we progress towards using more durable materials, we are committed to ensuring that all parts are either recyclable or disposed of in an environmentally responsible manner. Special attention is paid to aquatic-safe components to avoid potentially harming water environments.

Furthermore, our device includes a failsafe against overheating, enhancing safety while considering environmental implications. Future project iterations may explore using renewable energy sources, like solar power, to reduce environmental impact further.

3.1.3 Social Impact Design Considerations

The optogenetic device aims to make advanced research tools more accessible, potentially lowering entry barriers for educational institutions and small research labs. This accessibility could enrich scientific study and education by allowing a wider audience to engage in regenerative medicine and biological research.

Focusing on health, the device aims to support studies that could lead to tissue repair and regeneration breakthroughs. Such advancements could directly apply to medical treatments, offering new hope for patients with injuries or degenerative conditions.

In education, the device offers a tangible way for students to learn about optogenetics and regeneration, encouraging active engagement and sparking interest in bioscience careers. By simplifying complex research methods, it makes science more approachable and comprehensible.

The project acknowledges ethical considerations surrounding the use of live subjects in research and commits to adhering to established ethical standards in biological studies. An integrated temperature sensor in the design connects to automatically shut off when conditions become undesirable, ensuring the maintenance of safe conditions. The design's ease of repair and upgrade extends its lifespan and supports skill development in electronics and manufacturing, stimulating job growth in these sectors. Overall, through these design choices, the project aims to positively impact education, health, and employment while upholding ethical research practices.

3.1.4 Economic Design Considerations

The effective management of our $2000 budget is integral to the success of our optogenetic lightbox prototype. A meticulous breakdown of material costs, encompassing key components such as Arduino elements, LEDs, temperature sensors, displays, and housing materials, ensures transparency and facilitates judicious decision-making throughout the design process.

A detailed manufacturing estimate, incorporating a comprehensive Bill of Materials (BoM), is pivotal for economic considerations. A detailed BoM can be found in Appendix 1, detailing the cost breakdown of our various components. This systematic breakdown allows for a thorough assessment of the cost associated with each component, enabling informed decision-making and efficient budget utilization. Furthermore, a holistic cost estimation, encompassing materials, manufacturing, and prototyping, offers valuable insights into the economic landscape of the project. A detailed economic comparison with existing solutions illuminates our optogenetic lightbox's potential cost savings, efficiency gains, and other economic benefits.

Aligned with our primary objective of assisting Dr. Josh Currie's research, our project holds significant economic implications within the field of regenerative medicine. The potential insights into tissue repair and regeneration promise groundbreaking treatments, potentially mitigating healthcare costs associated with surgeries and prosthetics. A comparative analysis with prevailing market costs, which often exceed $10,000, underscores the potential cost-effectiveness of our innovative solution.

3.1.5 Failure Modes and Effects Analysis

The design's primary potential failure modes include electrical failures in LED operation, overheating of components, and structural integrity issues of the prototype housing. LED failures might occur due to improper soldering, inadequate current flow, or overuse, leading to insufficient illumination. Overheating could result from continuous operation or a lack of adequate cooling mechanisms, affecting the device's longevity and safety. Structural failures could arise from the material choice for the prototype housing, possibly compromising the device's durability and functionality. See Table 7 for a breakdown of these failure modes and associated probabilities.

Table 7: *Failure Modes and Probability*

	Risk		
Probability	**Low**	**Moderate**	**High**
Low	Sensor Malfunction	Structural Integrity Issues	Water Damage
Moderate	Software Errors	LED Burnout	Power Supply Failure
High	Improper LED Positioning	Diffuser Sheet Degradation	Electronic Circuitry Failure

Several improvements have been implemented to mitigate potential failures of the LumiGen Platform. These include using reliable power supplies with protective circuits, choosing high-quality electronic components, and incorporating modular components to prevent and repair LED burnout. The structural integrity of the device is enhanced by using durable materials like acrylic, which facilitates easy assembly and maintenance. Software reliability is ensured through rigorous testing and regular updates. LED positioning is carefully calibrated during initial setup, and the durability of the diffuser sheet is maintained by selecting robust materials and keeping periodic replacements accessible. Regular testing and maintenance protocols can also identify and rectify failures before they escalate. The design aims to enhance reliability, safety, and user experience through these strategies.

3.1.6 Other Embodiment Design Activities and Analyses

Finalizing the design for the alpha prototype primarily involved a focus on the material and component selection, as well as the system assembly. For the material selection, we only had to consider what to use for designing the main body. Cardboard was chosen, as it was inexpensive, readily available, and easy to work with. Any major changes could be corrected, and the body could be redone quickly as the structure of the components changed. For our major components, such as the LEDs, displays, temperature sensor, and microcontroller, we used a combination of tools that were already readily available. The Arduino, DHT-22, and all of the minor components (resistors, wires, buttons, etc.) were taken from our available resources. The other components, such as the power source, LEDs, switches, and wiring (power splitter) were found using external sources. Initial calculations, using equations from section 2.3, allowed us to determine the power needed from the LEDs to obtain an output close to 2 W/cm^2. This value is based on information found in the literature review, section 1.3.2. The assembly of the body was initially modeled in 3D software, before having individual parts represented in the laser cutter software. These files will be saved, and modified for the next phase of prototyping. The LED setup, pictured in section 3.1.7, was assembled so that it could be programmed as a single LED.

3.1.7 Preliminary Design Details

After careful consideration and planning for the design in the embodiment phase, the next step was to create the alpha prototype. The project method for building the alpha prototype can be divided into the cardboard body and the electrical circuit. Figures 13 and 14 are the CAD models that will be materialized into cardboard. The dimensions will be kept the same. Cardboard has the advantage of being cheap and close to real life because before expensive prototypes are created, cardboard workshops strengthen the understanding of the design without overspending and wasting acrylic material.

The electrical circuit consists of the LED array and the Arduino sub-circuit. A spool of ALITOVE 16.4ft 5050 SMD LED strips in red and blue were cut into equal lengths of 10 inches, soldered together in series (13 strips/length), and adhered to a cardboard surface. The displays, timer, and sensor were already functional from the embodiment design phase and will be combined with the LEDs to be programmed under one Arduino microcontroller. Figure 18 in Section 3.2 shows the schematic of the Arduino circuit, which includes the displays, timer, DHT-22 sensor, and the buttons to control the timer. The final step is to update the Arduino code to control the LEDs and the breadboard components.

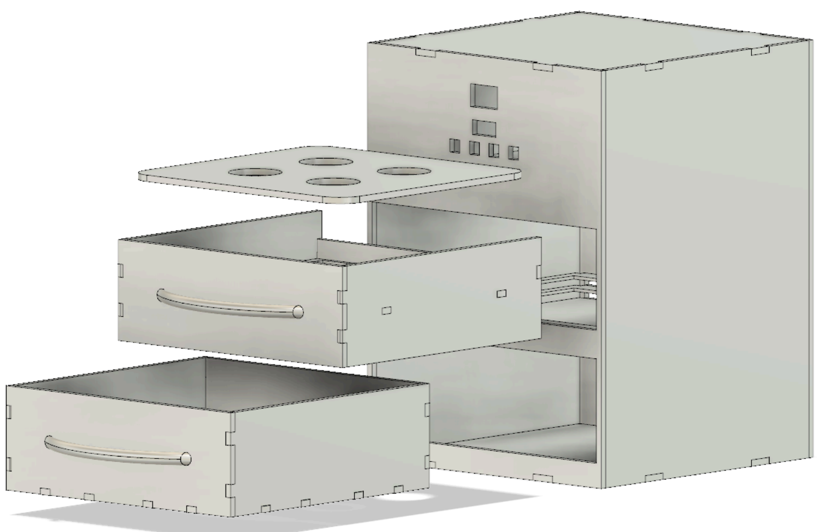

Figure 13. CAD design of the entire body

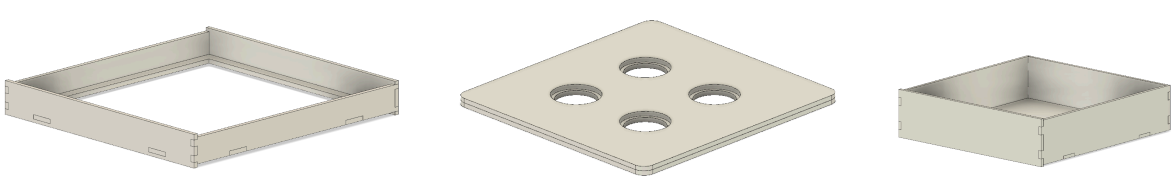

Figure 14. CAD structure of axolotl containment drawer

3.2 Alpha Prototyping

We used CAD models, the Boss Laser LS-1630 laser cutter, and plenty of corrugated cardboard as the main tools to create the body of the light box. Before laser cutting, we adjusted the model of the light box, including everything in the interior, to accommodate the thickness of the cardboard. Cardboard has a thickness of about 2 mm, while the acrylic PMMA sheets that will be used for the final prototype are about 6 mm. This adjustment was crucial to ensure that the tabs and slots fit, the drawers function properly without any friction, and that testing for the functionality of the design is smoother.

The next step involved exporting DXF files of the surfaces to make them compatible with LighBurn, the software used for laser cutting. The Wake Forest Engineering Department provided an ample supply of cardboard for the RegENineers to use in laser cutting. After cutting all the cardboard, we assembled the pieces for the body, drawers, and tray, adhering them together using hot glue. Finally, we 3D-printed handles and screwed them to the front of the drawers.

Before assembling the LED array, we cut cardboard trays using the laser cutter, which is where we will adhere to the LED strips. The trays will then be inserted into the body from the backside. To make the LED arrays, we started by cutting the LED strips into equal lengths for red and blue. We connected each color strip in series by soldering wires. This allowed us to turn the LED strips 180 degrees without bending the strips themselves. The layout of the LED strips inside the cardboard trays, with one for the top and one for the bottom to illuminate both sides, can be seen in a schematic in Figure 15. The wiring for the series connection can be seen in Figure 16. The last component was making the diffuser sheet frame out of cardboard, which is inserted in a tray above the LED strips with a guided railing.

For the rest of the circuit, we simply combined the LEDs with the sensors and timer into one Arduino microcontroller, using a single code to control all of the electrical components. This schematic can be seen in Figure 17.

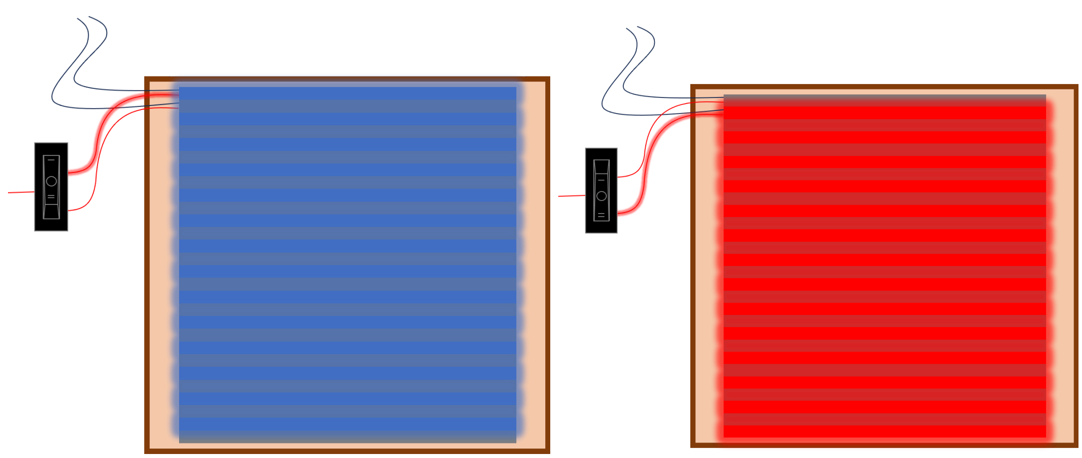

Figure 15. *Example of LED board switch capability*

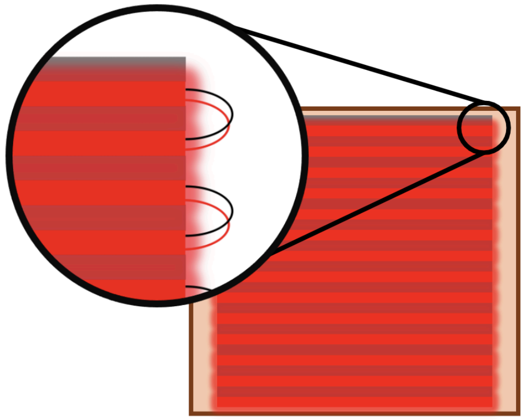

Figure 16. *Wiring diagram of LED side-by-sides*

For the rest of the circuit, we simply combined the LEDs with the sensors and timer into one Arduino microcontroller, using a single code to control all of the electrical components. The Arduino controls the PWM of LEDs to allow for blinking. A simple schematic of the powering of the LED strips and Arduino can be seen in Figure 17—the rocker switch changes which color is turned on and the power splitter is used to divide the flow of current into two paths to allow voltage to be the same across the Arduino and LEDs. The Arduino is able to handle up to 20 V and the adjustable power supply will only supply up to 10 volts max, so damage to the Arduino is not a concern. Figure 18 shows the configuration of the displays and DHT22 sensors.

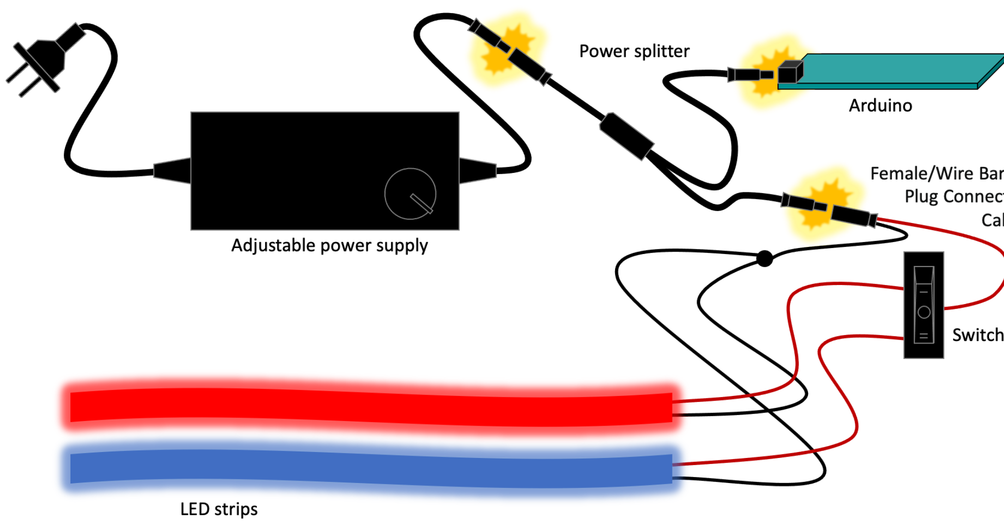

Figure 17. *Model of basic circuit structure*

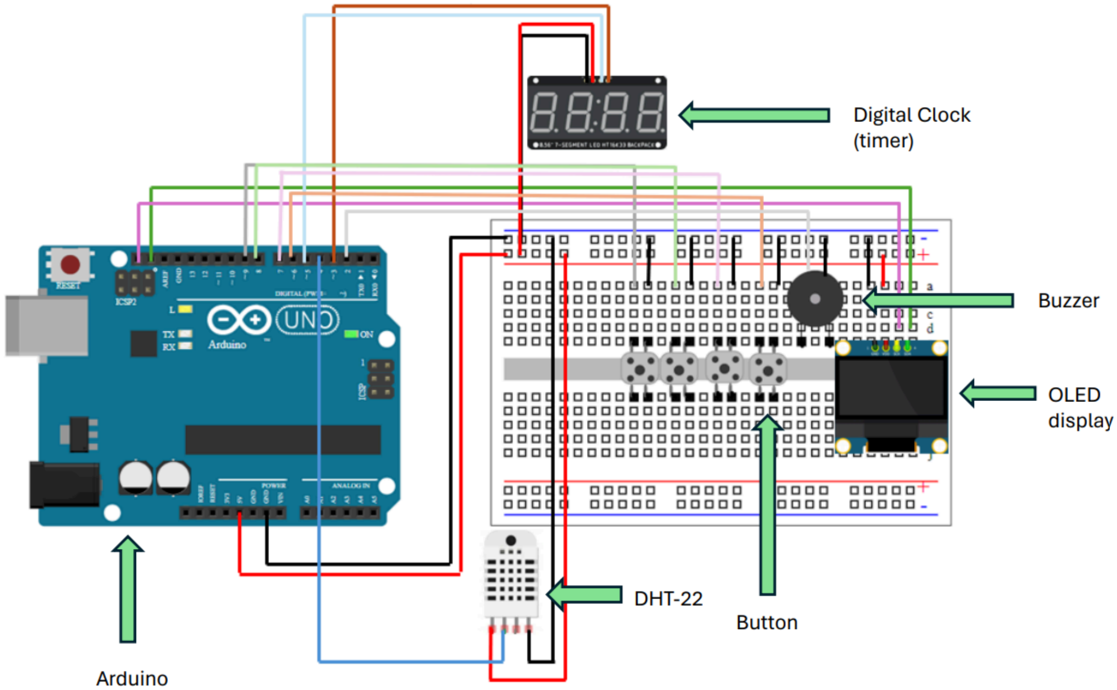

Figure 18. Schematic of the display setup

The final alpha prototype was a huge success in terms of physical functionality and dimensioning. The timer, sensors, displays, and LEDs worked perfectly on their own, however, we faced a small setback when we couldn't 100% combine the electrical components under one Arduino. The issue was that shortages were happening that we could not see or figure out yet, thus for this milestone we decided to keep the LEDs separate from the Arduino to prevent any risk of shortage and fires.

We have acquired valuable lessons and identified areas for enhancement through developing our alpha prototype. The construction process sharpened our soldering and circuit integration skills, underscoring the necessity for meticulous power management and current limitations to maintain system stability and prevent component damage. Our proficiency with laser cutting also evolved, enabling us to refine the manufacturability of our design for easier and more error-free assembly.

Using cardboard for the body was instrumental in visualizing the final device's structure, confirming the functionality of its mechanical design, and the operational success of individual components like LEDs, sensors, and displays (Figure 19). However, challenges arose in fully integrating these components into a cohesive system without causing damage. We plan to overcome this hurdle by reorganizing the circuitry, improving insulation, and refining the control code. Moreover, incorporating the electronic components into the prototype's body more seamlessly was a goal we fell short of achieving within our initial timeframe. For the beta prototype, we aim to ensure that all electrical systems are correctly embedded within the structure, enhancing the prototype's overall aesthetics and user interaction. We will transition from cardboard to acrylic for increased durability and a more refined appearance, and we are confident in our adjusted dimensions from the learnings of the alpha phase.

For the beta prototype, our primary objectives include refining the power supply system for greater reliability, optimizing wiring arrangements to eliminate potential faults, and enhancing the integration of LEDs with the control system to provide a more stable and consistent user experience. These improvements will address the feedback and challenges faced during the alpha prototype's development, leading us to a more polished and functional final prototype.

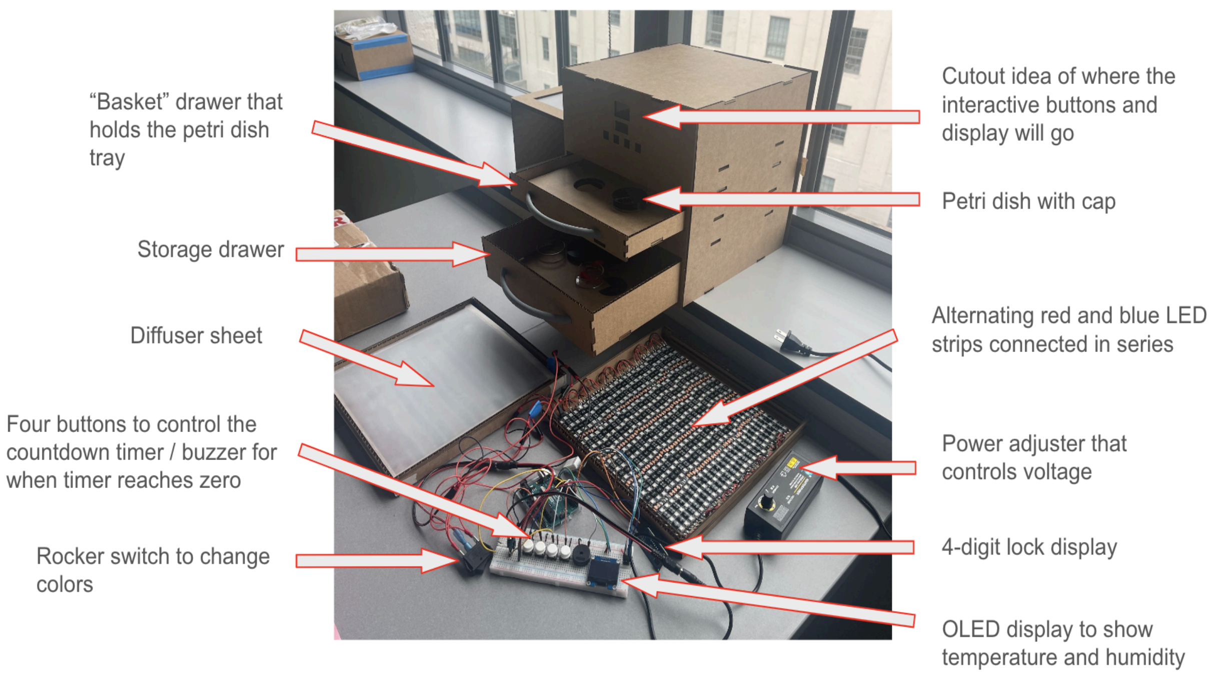

Figure 19. *Final alpha prototype with working LED array, sensors, and timer*

3.3 Preliminary Testing & Performance Evaluation

For the preliminary testing and performance evaluation of our optogenetic device prototype, we implemented a structured approach to assess the effectiveness of our LED illumination system. The core objective was to verify if the illuminance produced by our LEDs, when filtered through a diffuser sheet, met the project's specified system requirement of 1 mW per square centimeter, crucial for the optogenetic activation of proteins within axolotl specimens. To look at specific testing data and the associated calculations, reference Appendix 2.

Our testing procedure involved:

- Using a Thorlabs S121C Standard Photodiode Power Sensor, capable of measuring wavelengths from 400 to 1100 nm, to accurately capture illuminance levels produced by our LED setup.
- Conducting measurements in a controlled darkroom environment to eliminate external light interference and ensure accuracy.
- Employing a precise laser-cut structure designed to hold the diffuser sheet at predetermined distances (marked every ½ inch), allowing for systematic and replicable testing conditions.
- Placing the sensor directly beneath the diffuser sheet to measure illuminance at these various distances. The sensor's face size was 9.7 mm by 9.7 mm, enabling us to convert these readings to illuminance per area (cm^2).

The initial round of testing, averaging the results of three trials, showed that the illuminance achieved by our LED setup with a single diffuser sheet was approximately 1.4 mW per square centimeter for blue light and 0.8 mW per square centimeter for red light (Table 8). While these values indicated a shortfall from our target when using a single sheet, our design utilizes bidirectional illumination with two diffuser sheets. This configuration doubles the effective illuminance, leading to a combined output that achieves 140% of the required amount for maximal activation of the targeted proteins. Thus, our system exceeds the optimal parameters for optogenetic activation by leveraging the dual-sheet setup.

Table 8. *LED Testing Results Summary*

Voltage (V)	Color	Illuminance at 0.5 in (mW/cm^2)	Meets Desired Range (1+ mW/cm^2)
8	Blue	N/A	N/A
8	Red	0.492	No
9	Blue	0.735	No
9	Red	0.692	No
10	Blue	1.517	Yes
10	Red	0.933	No
11	Blue	2.301	Yes
11	Red	1.152	Yes
12	Blue	3.088	Yes
12	Red	1.436	Yes

We derived our voltage requirement of 10 V from these results. We thus meet our technical objectives with the current power source. Incorporating different light sources into further iterations of this device will require revisiting our hardware specifications and potentially adjusting our supplied voltage to accommodate different wavelengths. This iterative testing, analysis, and design modification process is pivotal in evolving our prototype to meet all specified system requirements and achieve our project goals.

4 DETAILED DESIGN

4.1 Design Improvements

During the Detailed Design phase, we refined our initial design by focusing on incorporating feedback and results from the Embodiment Design phase testing. Each improvement was carefully chosen to enhance the usability, reliability, and functionality of the LumiGen platform.

We replaced the existing drawer slides with an alternative that has a soft-lock feature, improving structural integrity and minimizing light escape by ensuring the drawers are closed flush with the device's face. We also narrowed the drawers to accommodate the width of the drawer slides on each side.

We also transitioned from a traditional breadboard setup to a custom-designed printed circuit board (PCB) from Adafruit, making our system more robust and less prone to wiring errors. This PCB effectively simulates a breadboard's connectivity but in a more compact and durable form, making the design more modular and the wiring slightly less complicated. We integrated a MOSFET to amplify the voltage supplied from the Arduino to the LEDs, ensuring consistent light output necessary for our optogenetic experiments. Lastly, we incorporated a feature to display the current wavelength of the LEDs on the OLED, addressing one of our stakeholders' primary concerns and facilitating the analysis of the axolotls' regenerative properties.

We enhanced the user interface to increase ease of use and to accommodate rigorous laboratory conditions. The front face was redesigned to include double-layered acrylic sheeting, providing a sturdy mount for electronic displays and operational buttons, enhancing both aesthetic and functional aspects of the LumiGen platform.

The adjustments and enhancements have rendered the LumiGen platform more efficient and adaptable, simplifying maintenance and ensuring it can reliably support cutting-edge research in regenerative medicine.

4.2 Beta Prototype

With these design improvements, we produced our beta prototype (Figure 20). We began to construct our acrylic frame (excluding the front and back walls). We also made small adjustments to our circuitry and wiring, which are detailed below.

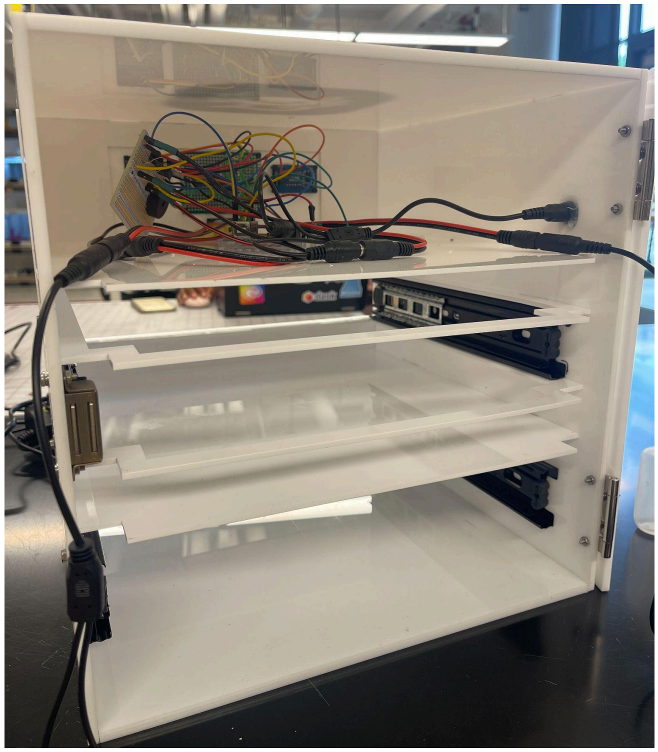

Figure 20. *Beta prototype. Front wall needs to be finalized to accommodate proper displays at this stage, and the back needs to be adapted to be accessible.*

<u>Button and Displays</u>

Using Fusion 360, the buttons and displays were measured to scale and added to the previous CAD model to provide a visual representation of what the front would look like (Figure 21). This layout ended up being different later in the final design, but this step assisted in figuring out what would be practical and appealing for the user.

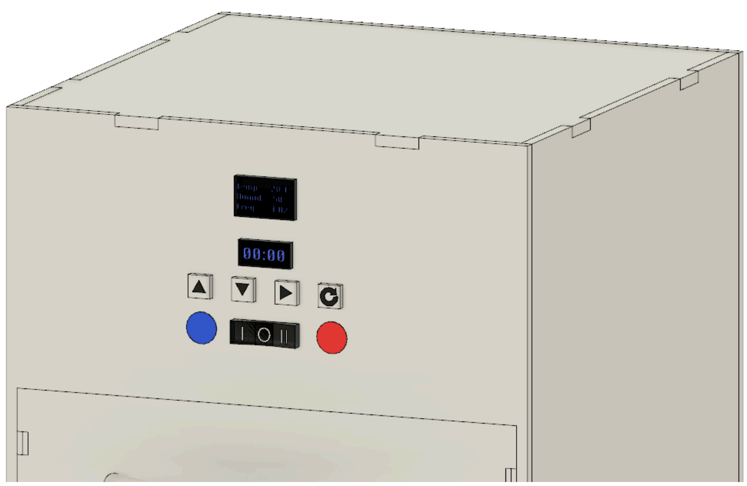

Figure 21. *Final CAD model after including the visuals for the interactive buttons and displays*

Petri Dish Caps and Bases

Originally, the caps and bases were shaped as major sectors with 90° angle minor sector cutouts. These components can still be used for experiments but the issue is that the openings are too wide, which would not 100% fulfill the requirement of targeted limb illumination. Thus, the new caps and bases are designed to have smaller holes to better isolate the specific part of the specimens that the user wants to illuminate. There are a variety of designs from circles and squares to the holes being located in the center or edge to assist in the targeting (Figure 22a). Additionally, underneath the new caps, internal walls extend from the edge of the holes to make the path of the light more direct to the specific target as shown in Figure 22b.

Figure 22a. *Original caps on the top row with the new designs in the bottom row*

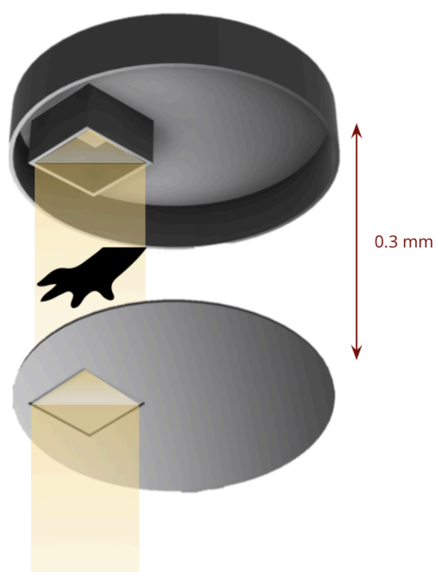

Figure 22b. *Internal walls underneath the cap reduce scatter of light*

LED wiring

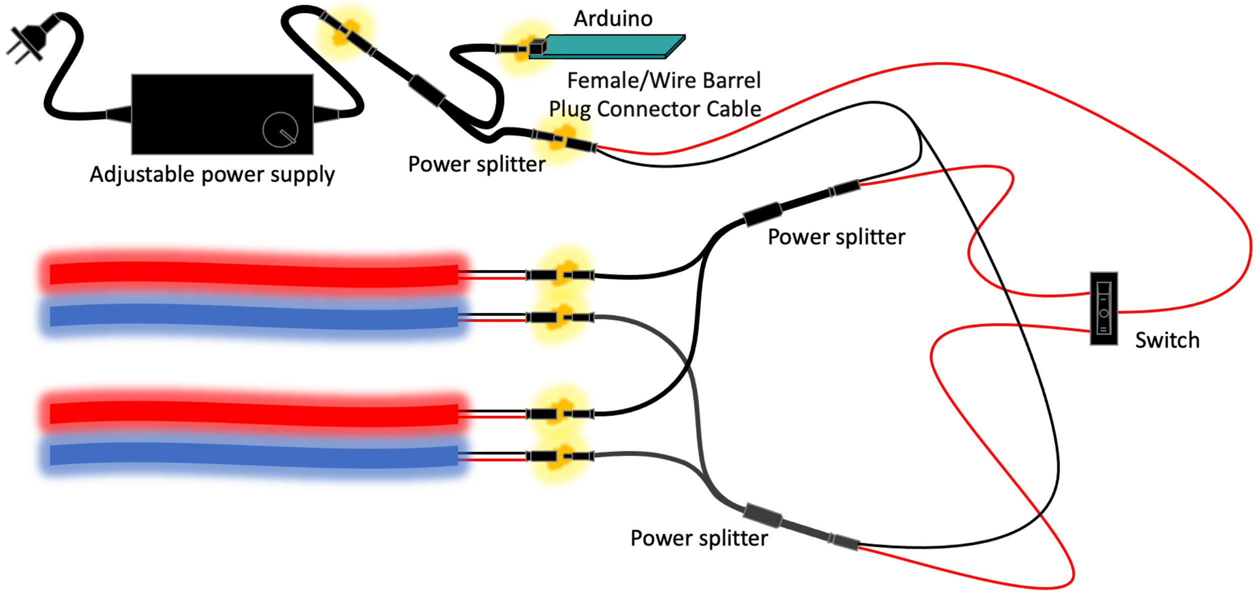

Figure 23. *Updated wiring of LEDs for beta prototype. Includes more power splitters and male/female connections.*

The LED wiring was updated as shown in Figure 23. Component functionality remained the same, but power splitters were integrated to allow for greater accessibility in case light strips needed to be replaced.

Final circuit design

The final circuit has been made to have three different settings for the wavelength selection. These include frequencies of 0 ms, 500 ms, and 1000 ms, with 0 ms being just a constant non-pulsing light. This is controlled with a single, additional button. A second PCB was also added solely for the wiring of the buttons, and to make it simpler to attach to the face of the device. In Figure 24 below, the connections between these components can be seen. The MOSFET is to be connected directly to the LED displays. It includes an additional 1000 Ohm resistor that is used to protect it from damage while still allowing it to amplify the voltage to the LEDs. Lastly, the timer has been changed from a minute-second format to an hour-minute format, as this is another feature that would increase the convenience of our device for the very particular needs of our stakeholder.

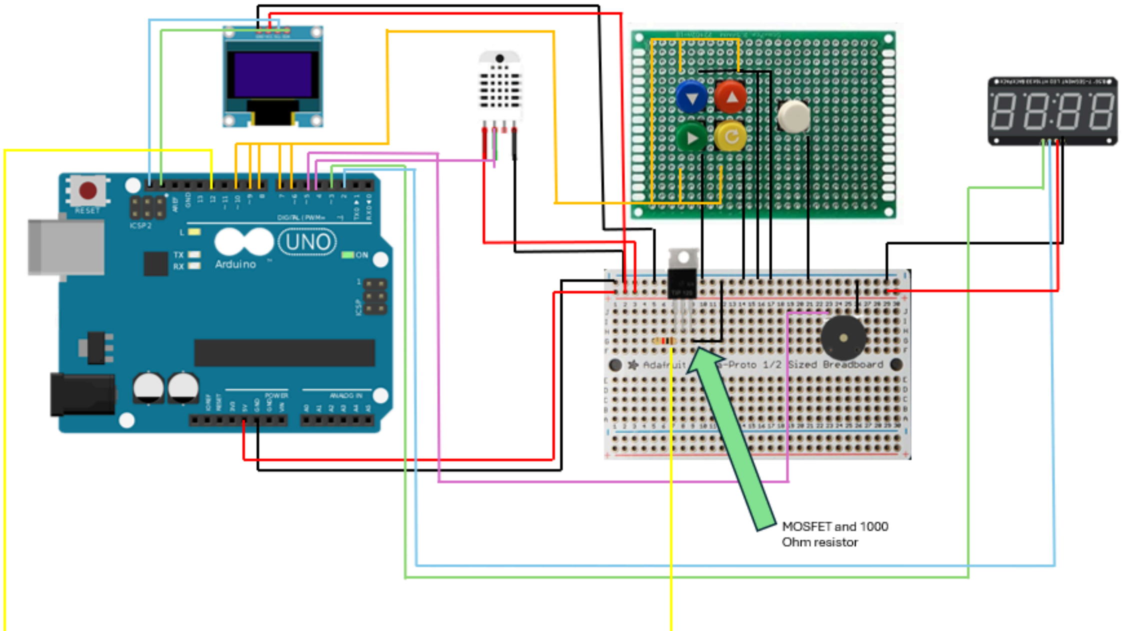

Figure 24. *Updated circuit design with additional connections accounting for the 2 PCBs, MOSFET, tactile buttons, and 1000 Ohm resistor.*

4.3 Testing & Performance Evaluation for the Detailed Design

During the development of our prototype, significant emphasis was placed on refining both the LED array and the placement of the DHT22 sensor to enhance functionality and ensure operational safety. Initial integration of the LED array with the Arduino involved rigorous electrical testing to determine the appropriate voltage and current levels. The forward voltage for blue LEDs is higher than red because blue light has a higher frequency (shorter wavelengths). However, LEDs are not voltage-driven devices – they are current-driven. The goal was to have a nominal current under 2A. A current higher than that value would damage the LEDs and age them prematurely. Using a multimeter, the current for the red strips was about 1.1 A and about 0.18 A for the blue strips. The voltage across the strips, when the lights turn on, is about 6.35 V for the red strips, and about 7.97 V for the blue strips.

Additionally, the positioning of the DHT22 sensor was optimized to enhance the accuracy of environmental monitoring. Initially placed at the edge of the petri dish platform, we relocated the sensor to the middle to better capture the ambient conditions affecting the specimens. This adjustment was part of our move towards a more modular system design, which also facilitated the smooth full extension of the drawer containing the petri dishes. This modular approach allowed for easy removal and replacement of components, significantly improving maintenance efficiency and operational convenience. These enhancements directly addressed our system requirements and were critical in refining the prototype's design to better serve the needs of the Currie laboratory's research into axolotl regeneration.

4.4 Final Prototype

The final iteration of the LumiGen Platform (Figure 25) showcases significant advancements in both functionality and design, aligning closely with the project's objectives and the specific needs of the Currie laboratory. This version incorporates a robust construction with reinforced front and back walls which were meticulously tested to ensure durability and effective operation. The integration of magnets and hinges enhances the accessibility and maintenance of internal components, allowing for easier adjustments and replacements.

Figure 25. *In order: isometric view, top view, and view inside the basket drawer*

In terms of operational controls, the LumiGen Platform features an intuitive interface with a timer, buttons for timer adjustment (blue to decrease time, red to increase, green to start/pause, and yellow to reset), and a white button to toggle pulse modulation (Figure 26). This setup is clearly displayed on the front panel, ensuring user-friendly interaction. Additionally, a switch allows for a seamless transition between blue and red light settings, corresponding to the specific wavelengths required for activating or inhibiting gene expression in axolotl studies. Final code can be found in Appendix 3.

Figure 26. *Final prototype with display. Moving left to right, we have the timer, buttons to set the timer (blue: decreases time, red: increases time, green: start/pause, yellow: reset), a white button to change pulse modulation, and our display. Below is the switch to control between blue (I) and red (II) light.*

While the electrical circuitry has remained largely unchanged from the beta prototype, its reliability and effectiveness in delivering precise light control have been validated through rigorous testing. This final design successfully meets the rigorous standards and system requirements set forth at the project's inception, efficiently catering to the nuanced demands of regenerative research.

Despite the achievements of the LumiGen Platform, there is potential for further enhancements. One area for improvement would be the incorporation of additional light settings to accommodate a broader range of experimental needs. This would allow researchers to conduct more specialized studies with varying light intensities and wavelengths, potentially broadening the scope of research applications. These enhancements could provide even greater flexibility and precision in future iterations of the device, furthering its contribution to the field of regenerative medicine.

4.5 Commercialization

We do not plan to commercialize our product on a larger scale since we have designed it with specific research in mind. Ideally, we need more time to test the quality of the LumiGen Platform in a lab setting before shifting to commercialization. However, there is a huge potential for making our device into a successful product. We know that optogenetic researchers and universities would be our most sought-after customers as our device is the only non-invasive instrument that can house macroorganisms in a semi-freely behaving environment. The immediate next step would be to showcase the platform's core capabilities and features, allowing for testing and feedback from potential users. Validation and testing are paramount to ensure the platform meets scientific standards, accuracy requirements, and regulatory compliance. Intellectual property protection through a patent is also critical to safeguard the platform's uniqueness and claim credit for our idea.

As discussed previously, throughout the process of building the platform, regulatory compliance has been thoroughly understood and adhered to through our stakeholder, Dr. Josh Currie. The regulatory compliance covers the care of research axolotls and includes guidelines for the oversight and conduct of studies intended to minimize or prevent pain or distress of the laboratory animals.

Once we pass validation and testing, our strategy would be to explore partnerships and collaborations with research institutions, universities, or industry partners that can provide valuable resources, expertise, and access to a broader customer base. They could help build a business model that encompasses pricing strategies, revenue streams, distribution channels, customer support mechanisms, and robust marketing and sales strategies that are essential for sustainable commercialization.

For now, our focus is on accessibility, so we have designed a GitHub so that any university seeking to perform similar research could construct our device without needing specialized equipment or materials to ensure users understand how to utilize the platform optimally and address any issues they may encounter.

5 PROJECT MANAGEMENT

5.1 Changes Implemented Since the most recent Design Review

Since the most recent Design Review, on Friday, April 5th, we had begun putting more emphasis on the development of the alpha prototype. Each of the major components, including the body, display setup, and LED setup had undergone significant changes from our initial design.

While the body for this phase of the prototype was still made from cardboard, it had been changed to include two separate compartments. One holds the specimen and LED setup, while the other is simply for storage. The design has also been made more specific for use in the laser cutter, with all of the edges being interlocking to make the assembly easier.

Similarly, the LEDs had to be wired together to fit into a tray that would go within the main body. They were wired in a way that would allow them to each be programmed as individual LEDs. Two trays had been included in the design to hold the red and blue LEDs separately.

The most significant of these changes was the decision to connect the Arduino from the display setup to the LED setup. This was to control the interval of pulsing, an important feature that is needed for activating the proteins that will allow our stakeholders to analyze the regenerative properties of the specimen. This also caused the greatest challenge, as the current display setup was not providing enough voltage to switch on the LEDs. We had included a MOSFET into the circuit to amplify the voltage traveling to the LEDs. When connecting the LED setup to the full display circuit, the circuit would short and shut off all of the components. One notable aspect of this was that the MOSFET had been fried during one of these instances. While we have not yet diagnosed the cause of this, it has been suggested that it may have to do with the 220 mA current on the Arduino.

We had begun testing the output of the LEDs in terms of illuminance and power. They had shown that the current LEDs do not meet the requirements for producing the 2 Watts/cm^2 needed in our stakeholder's experiments until we took the bidirectional illumination into consideration. With this in mind, we found that 10 V from our power source was appropriate to produce our desired illuminance.

We also created a GitHub page that would be used to store the build instructions for our completed design. This would also serve as a guide for repairs in the future. A QR sticker code is found on the side of the main body for access.

6 CONCLUSIONS AND RECOMMENDATIONS

The LumiGen Platform stands as a testament to our team's ingenuity and dedication, representing a significant milestone in optogenetics. Specifically tailored for the study of axolotl regeneration, this project has met the established system requirements, delivering precise control over light exposure in terms of wavelength and intensity. This precision is pivotal in manipulating light-sensitive proteins crucial for tissue regeneration.

The device functioned effectively, delivering controlled illumination to targeted areas, which allowed for detailed examination of regenerative processes under varying conditions. This capability supported the Currie laboratory's research and set a foundation for broader applications in regenerative medicine. The platform's design facilitates consistent, repeatable experiments, a critical factor for scientific research, which has been well-received by the customer, affirming their satisfaction with the device's functionality and user interface.

Looking ahead, the LumiGen Platform has the potential to make a substantial impact on the field. By enabling precise genetic control through light, this device assists researchers in unraveling the complex biological mechanisms behind tissue regeneration and repair. This could lead to breakthroughs that extend beyond axolotls, potentially informing therapeutic strategies for human medicine. To enhance the design further, several improvements could be considered such as interface enhancements, increased adjustability, and expanding the available light spectrum. Updating the buttons and displays for better ergonomics and integration could enhance user experience and reduce potential operational errors, while incorporating additional light wavelengths would enable the device to support a wider range of experiments, increasing its utility and adaptability. Lastly, Improving the adjustability of light intensity and exposure areas would allow for more nuanced experiments, catering to more specific research needs.

As for commercialization potential, the LumiGen Platform stands as a promising candidate for wider adoption in academic and clinical research settings. The transition from a prototype to a market-ready product would involve scaling up manufacturing processes, standardizing components for reliability, and ensuring compliance with relevant regulatory standards. Challenges might include securing funding for mass production, achieving cost-efficiency without compromising quality, and navigating the regulatory landscape associated with medical research tools.

Continued development and testing are recommended to refine the design and extend its capabilities. Future work could explore integration with automated data collection and analysis systems, enhancing the platform's functionality and making it even more attractive for commercialization. The pathway to market will require a detailed business plan, partnerships with biomedical companies, and further prototyping to meet wider industry standards.

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The RegENgineers: Sarah, Kyle, John, and Elena

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8 Appendix 1: Bill of Materials

Pictures of our final Bill of Materials can be found below. Link here.

Component	Specific part (model)	Link	Do we have it?	Quantity	Cost/unit	Total cost	Notes	Justification
Arduino	Arduino uno	Arduino Uno site	Available for prototype	1	$27.60	$27.60	Ask Di Vittorio: do we get this for free? Look at the different arduinos and see if one would be better suited than the uno. Uno justification: simplicity of use, we're familiar with it. We can come up with more as needed. Make sure that we meet the current requirement (200 mA).	
460 nm LED	LED strip	Blue LED	Purchased, here	2	$12.99	$25.98	https://www.instructables.com/Intro-to-LED-Strips/	
660 nm LED	LED strip	Red LED	Purchased, here	2	$12.99	$25.98		
Temperature sensing (DHT 22)	DHT 22 temperature-humidity sensor	DHT22 x3	Purchased, here	1	$12.99	$12.99	Can borrow from EGR dept. for testing., set of 3 with current link	
Arduino power supply	Adjustable Power Supply with 2.1mm / 5.5mm DC - 3V to 12V at 5A	Adjustible supply	Purchased, here	1	$17.50	$17.50		
OLED Display (temperature)	HiLetgo 1.3" IIC I2C Serial 128x64 SSH1106 SSD1306 OLED LCD Display LCD Module for Arduino AVR PIC STM32	OLED Display	Purchased, here	3	$8.49	$25.47		
4-digit Display (timer)	4 digit clock display	4 digit display	Purchased, here	1	$7.99	$7.99		
White PMMA Acrylic (1/4", 18x24")	1/4" white acrylic sheets, 18x24	https://makerstock.c	Purchased, here	8	$26.95	$25.47		
White PMMA Acrylic (1/4", 12x24")	1/4" white acrylic sheets, 12x24	https://makerstock.c	Purchased, here	10	$17.95	$179.50		
White PMMA Acrylic (1/8", 18x24")	1/8" white acrylic sheets, 18x24	https://makerstock.c	Purchased, here	8	$16.95	$25.47		
White PMMA Acrylic (1/8", 12x24")	1/8" white acrylic sheets , 12x24	https://makerstock.c	Purchased, here	4	$11.95	$47.80		
Petri dishes	Pyrex glass petri dishes	Petri culture dishes	Available for prototype	1	$10.45	$10.45	60 x 15mm glass petri dishes (provided by Currie?), using the cap	We chose to go with glass plates to reduce light distortion. We chose these specific ones because they are what Dr. Currie uses in his lab.
Wavelength control switch	3 position rocker switch	Rocker switch x5	Purchased, here	1	$7.99	$7.99		
Diffuser sheet	Light diffusing sheet	Light Diffusers	Purchased, here	1	$15.98	$15.98		
Crimping tool	Wirefy Crimping Tool	Crimper	Purchased, here	1	$24.99	$24.99		
Spade connectors	Feggizuli 280PCS Crimp Connectors	Spade Connectors	Purchased, here	1	$8.98	$8.98		
DC power pigtail cable	16AWG DC Barrel Plug Connector 2.1mm x 5.5mm DC Male Plug to Bare Wire Open End Power Wire Supply Repair Cord	DC Power Pigtail	Purchased, here	2	$7.99	$15.98		
Lux sensor	Light Meter Digital Illuminance Meter	Lux Sensor	Purchased, here	1	$25.99	$25.99		
Epoxy drawer slides	Builders Line White Epoxy Coated Euro Drawer Slide	slide drawers	Purchased, here	4	$2.33	$9.32	10 inch drawer slides for the basket (**DID NOT USE**)	easy to install and helps with stability and smoothness
Metal drawer slides	Full Extension Less Noise Ball Bearir	drawers	Purchased, here	1	$29.99	$29.99		
Rocker Switches	ON/Off/ON Toggle Rocker Switch	Switch	Purchased, here	1	$7.99	$7.99		
L-bracket corner brace set	50 brackets and 100 screw	L-brackets	Purchased, here	1	$6.78	$6.78		
Cabinet Magnets	Pack Cabinet Magnetic Catch Cupboard	magnet	Purchased, here	1	$6.99	$6.99	For a door latch - makes it so the back is accessable	
Door Hinges	4 pack of hinges	hinges	Purchased, here	1	$7.99	$7.99	For a door latch - makes it so the back is accessable	
Powet Splitter	1 to 2 Way DC Power Splitter Cable Barrel Plug	power splitter	Purchased, here	2	$6.12	$12.24		
Male and female barrel connectors	10 Pairs 12V 5A DC Power Pigtail Barrel Plug Connector Cable	barrel connectors	Purchased, here	1	$9.29	$9.29		
Relay module	HiLetgo 2pcs 5V One Channel Relay Module Relay Switch	Module	Purchased, here	1	$7.39	$7.39	DID NOT USE	
Breadboard PCB	Adafruit Perma-Proto Half-sized Breadboard PCB - 3 Pack!	BB PCB	Purchased, here	1	$12.50	$12.50		
stranded wires	stranded copper wire	stranded wires	Available for prototype	1	$16.95	$12.50	used for the DHT-22 connections for flexability	
solid core wires	24 AWG Solid Core Wire Kit	solid wires	Available for prototype	1	$14.94	$14.94		
Push buttons	Adafruit Round Tactile Button Switch, pack of 15	push buttons	Available for prototype	1	$5.95	$5.95		
heat shrink tubing	color assorted heat shrink tubing	https://www.amazon	Available for prototype	1	$6.99	$6.99		
dupont connectors	Dupont Connector Kit 2.54mm	DuPont	Available for prototype	1	$9.99	$9.99	just need the single connectors	
Grey resin	Formlabs Grey Resin v4		Available for prototype			0	It's very nice and durable material but quite expensive and obviously not available everywhere. you can also use FDM 3D printer to make handles	used to make the handles for the ligh box
Weld-On #4	SCIGRIP Weld-On #4 Adhesive (4 oz.)	weld-on #4	Available for prototype	1	$27.59	$27.59		used to weld the acrylic together
PLA filament	Black MH Build Series PLA Filament - 1.75mm (1kg)	black pla	Available for prototype	1	$20.87	$20.87	note that you must use 3D printer that is compatible with 1.75mm thickness	used to make the petri dish caps and filters
MOSFET	n-type enhancement type	mosfet	Available for prototype	1	$2.79	$2.79		
PCB board	2x3" board	PCB	Available for prototype	1	$3.29	$3.29		

9 Appendix 2: Data Collection

Pictures of our Data Collection Efforts can be found below. Link here. An example of the layout can be found below with the blue light data.

Test 4: LED Array (Blue) - 11 Volts			
Distance (in)	Illuminance (mW)	Illuminance (mW) per cm^2	Desired Range: 1+ mW/cm^2
0.5	0.851	1.15093495	2.3018699
1	0.816	1.1035992	2.2071984
1.5	0.742	1.0035179	2.0070358
2	0.629	0.85069105	1.7013821
2.5	0.512	0.6924544	1.3849088

Test 5: LED Array (Blue) - 12 Volts			
Distance (in)	Illuminance (mW)	Illuminance (mW) per cm^2	Desired Range: 1+ mW/cm^2
0.5	1.142	1.5444979	3.0889958
1	1.078	1.4579411	2.9158822
1.5	0.943	1.27536035	2.5507207
2	0.832	1.1252384	2.2504768
2.5	0.704	0.9521248	1.9042496

Sheets are organized based on raw data, thorlab sensor calculations, previous luminance calculations based on lux sensor readings, and mathematical justifications with sources.

Note	Source
The conversion of lux [lx] to watt/centimeter² (at 555 nm) [W/cm² (at 555 nm)]	Link
Luminous Efficacy Table	Link
Lux to Watt Calculator	Link

Math	Equation	Modified
Lux to watts calculation with area in square meters	P(W) = Ev(lux) x A (m^2) / η (lm/W)	P(W)/ A (m^2) = Ev (lux) / η (lm/W)

Blue Light	lm/W	Watts per cm^2 modifier
	15.709	0.00000636577758

Red Light	lm/W	Watts per cm^2 modifier
	41.663	0.000002400211219

10 Appendix 3: Code

```
#include <TM1637Display.h>  // Include TM1637 Display library
#include "U8glib.h"  // Include U8glib for OLED display
#include "DHT.h"  // Include DHT sensor library

// Define the connections pins to TM1637 and buzzer
#define CLK 2
#define DIO 3
#define BUZZER_PIN  5 // Define the pin connected to the buzzer

// Define button pins
#define BUTTON_SELECT 10 // for pulsing selection
#define BUTTON_INCREASE 8
#define BUTTON_DECREASE 9
#define BUTTON_START_STOP 7
#define BUTTON_RESET 6

// Create display object for TM1637
TM1637Display display(CLK, DIO);

// Define DHT sensor setup
#define DHTPIN 4  // Change as per your connection
#define DHTTYPE DHT22
DHT dht(DHTPIN, DHTTYPE);

// Create OLED display object
U8GLIB_SH1106_128X64 u8g(U8G_I2C_OPT_NONE);

// Variables to store time, temperature, and timer state
int timeHours = 0;
int timeMinutes = 0;
bool timerRunning = false;
String temp;
String humi;

// Variables for timing with millis() and LED control
unsigned long previousMillis = 0;
bool ledState = LOW;
unsigned long lastLedToggleTime = 0;
```

```
// Frequency Selection
int frequencies[] = {0, 500, 1000}; // Frequencies: 0 (constant), 500 ms,
1000 ms
int currentFrequency = 0; // Default to constant frequency

unsigned long buzzerEndTime = 0;
const unsigned long buzzerDuration = 20000; // 20 seconds for buzzer

void setup() {
  display.setBrightness(0x0f);
  updateTimeDisplay();

  dht.begin();

  pinMode(BUTTON_INCREASE, INPUT_PULLUP);
  pinMode(BUTTON_DECREASE, INPUT_PULLUP);
  pinMode(BUTTON_START_STOP, INPUT_PULLUP);
  pinMode(BUTTON_RESET, INPUT_PULLUP);
  pinMode(BUTTON_SELECT, INPUT_PULLUP);

  pinMode(BUZZER_PIN, OUTPUT);
  digitalWrite(BUZZER_PIN, LOW); // Ensure buzzer is off initially

  pinMode(12, OUTPUT); // Setup LED pin as an output
  digitalWrite(12, LOW); // Ensure LED is off initially

  Serial.begin(9600);
}

void loop() {
  unsigned long currentMillis = millis();

  if (timerRunning) {
    if (currentMillis - previousMillis >= 60000) {
      previousMillis = currentMillis;
      timerTick();
    }
    updateLed(currentMillis); // Update LED state while the timer is
running
```

```
  }

  if (digitalRead(BUTTON_INCREASE) == LOW) {
    increaseTime();
    delay(200); // Simple debounce
  }

  if (digitalRead(BUTTON_DECREASE) == LOW) {
    decreaseTime();
    delay(200); // Simple debounce
  }

  if (digitalRead(BUTTON_START_STOP) == LOW) {
    startStopTimer();
    delay(200); // Simple debounce
  }

  if (digitalRead(BUTTON_RESET) == LOW && !timerRunning) {
    resetTimer();
    delay(200); // Simple debounce
  }

  if (digitalRead(BUTTON_SELECT) == LOW) {
    currentFrequency = (currentFrequency + 1) % 3; // Cycle through
frequencies
    delay(200); // Debounce delay
  }

  float h = dht.readHumidity();
  float t = dht.readTemperature();
  temp = String(t);
  humi = String(h);

  if (!isnan(h) && !isnan(t)) {
    u8g.firstPage();
    do {
      draw();
    } while (u8g.nextPage());
  }
  delay(10);  // Short delay for stability
```

```
  // Manage the buzzer time-out
  if (buzzerEndTime > 0 && (millis() - buzzerEndTime >= buzzerDuration)) {
    digitalWrite(BUZZER_PIN, LOW);  // Turn off the buzzer after 20
seconds
    digitalWrite(12, LOW);  // Turn off the LEDs
    buzzerEndTime = 0;  // Reset the buzzer timer
  }
}

void updateTimeDisplay() {
  display.showNumberDecEx(timeHours * 100 + timeMinutes, 0b01000000,
true);
}

void increaseTime() {
  timeMinutes++;
  if (timeMinutes >= 60) {
    timeMinutes = 0;
    timeHours++;
    if (timeHours >= 24) {
      timeHours = 0;
    }
  }
  updateTimeDisplay();
}

void decreaseTime() {
  timeMinutes--;
  if (timeMinutes < 0) {
    timeMinutes = 59;
    timeHours--;
    if (timeHours < 0) {
      timeHours = 23;
    }
  }
  updateTimeDisplay();
}

void startStopTimer() {
```

```
  timerRunning = !timerRunning; // Toggle timer state
  if (timerRunning) {
    previousMillis = millis() - 60000; // Adjust time to count down
immediately
    digitalWrite(12, HIGH); // Turn on LED when timer starts
  } else {
    digitalWrite(12, LOW); // Turn off LED when timer stops
    buzzerEndTime = millis(); // Set buzzer to start time
    digitalWrite(BUZZER_PIN, HIGH); // Turn on buzzer
  }
}

void resetTimer() {
  timeHours = 0;
  timeMinutes = 0;
  updateTimeDisplay();
  digitalWrite(BUZZER_PIN, LOW);
  digitalWrite(12, LOW);
  timerRunning = false;


}

void timerTick() {
  if (timeMinutes > 0 || timeHours > 0) {
    timeMinutes--;
    if (timeMinutes < 0) {
      timeMinutes = 59;
      timeHours--;
    }
  } else {
    timerRunning = false;
    buzzerEndTime = millis(); // Set the time when buzzer started
  }
  updateTimeDisplay();
}

void draw() {
  u8g.setFont(u8g_font_unifont);
  u8g.drawStr(0, 11, "Temp:");
```

```
  u8g.setPrintPos(70, 11);
  u8g.print(temp + " C");
  u8g.drawStr(0, 28, "Humid:");
  u8g.setPrintPos(70, 28);
  u8g.print(humi + "%");
  u8g.drawStr(0, 45, "Freq (ms):");
  u8g.setPrintPos(70, 45);
  u8g.print(frequencies[currentFrequency]);
}

void updateLed(unsigned long currentMillis) {
  if (frequencies[currentFrequency] == 0) {
    digitalWrite(12, HIGH);
  } else {
    if (currentMillis - lastLedToggleTime >=
frequencies[currentFrequency]) {
      ledState = !ledState;
      digitalWrite(12, ledState);
      lastLedToggleTime = currentMillis;
    }
  }
}
```