Microscopic Cell Culture Incubator
Final Report

BME 200/300 Design
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Abstract

The team was tasked with creating and testing a cell culture incubator that will maintain a specific internal environment while being compatible with an inverted microscope. The internal environment must be 37°C, >95% humidity, and contain 5% CO_2 in the air. There are current designs on the market that meet this criteria, but the inverted microscope is encapsulated into the incubator making it bulky and inconvenient to disassemble. The team is going to design a cell culture incubator that will be portable and small enough to fit on the inverted microscope stage, allowing the user to view live cells inside of the incubator. The incubator will include a heated water pump and CO_2 pump in order to reach the clients criteria. Transparency, heating, and insulation testing will be conducted on various materials to find the optimal combination for the incubator.

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Body of Report

I. Introduction

A cell culture is a commonly practiced laboratory method for the use of studying cell biology, replicating disease mechanisms, and investigating drug compounds [1]. Due to the use of live cells during this process, incubators are necessary to keep the cells viable for the amount of time being studied. Incubators allow for live cell growth because they maintain a highly regulated internal environment of 37℃, 5% CO_2, and 95% humidity, without compromising the integrity of the microscope. The COVID-19 pandemic has allowed for the CO_2 incubator market to increase 7.69% with an estimated market growth acceleration of 8% over the next decade [2]. Major disadvantages of current commercially available systems are that they tend to be large and bulky enclosing the entirety of the microscope making it difficult to assemble and remove between uses, while also hindering the use of the microscope in general, and they are often expensive; Fisher Scientific's Enviro-Genie cell incubator is priced at $6,510.68 [3]. This project will focus on developing a low-cost cell culture incubator that allows for interchangeable culture plates, compatibility with an inverted microscope, easy disinfection, and live cell imaging via maintenance of the internal environment needed for cell growth.

II. Background

Cell Cultures in Lab

Cell cultures are mainly used in the study of cell biology due to their ability to easily manipulate genes, molecular pathways, and culture systems to remove interfering genetic and environmental variables [4]. Cell cultures follow BioSafety Level 2 [5], which describes the safety procedures for working in a lab that can be associated with human diseases, and any incubators being used in conjunction with cell cultures must follow ISO Class 5 air quality standards [6]. Cell cultures have the ability to work with three different cell types: primary, transformed, and self-renewing cells. Primary cells are directly isolated from human tissue. Transformed cells are those that can be generated naturally with changes to the genetic code, or genetically manipulated. Self-renewing cells are cells that carry the ability to differentiate into a variety of other cell types with long-term maintenance in vitro. An example of self-renewing cells are embryonic stem cells [1].

Incubators used in cell cultures have to maintain a very stable microenvironment and can achieve this via regulated temperature, CO_2, O_2, and pH levels. Controlling these factors is critical for the viability and growth of the cultured cells, as the incubator is aiming to replicate the cells' natural conditions (37℃ with a pH of 7.2-7.4) [7]. CO_2 is needed as a buffer to help with the pH along with a culture medium. The medium most commonly used is a Basal medium, with occasional serums added (such as fetal bovine serum), which controls the physicochemical properties of the cell cultures pH and cellular osmotic pressure [1].

Incubator Types

There are two types of commonly used methods to maintain temperature in industry cell incubators. Many employ the electric coils method which tends to give off heat through metal coils that surround the body of the incubator, programmed to the desired temperature. The other method is the water-jacketed incubators which use a controlled circulating water bath cabinet around the body of the incubator for even heating throughout the entirety of the chamber.

Humidity control is achieved most commonly by placing a tray of water at the bottom of the incubator. This method is used in both water jacketed and direct heat incubators. CO_2 control is achieved through a CO_2 tank that automatically pumps the desired amount of gas into the incubator. Using tubes and a valve connector, the CO_2 tank is able to deliver gas to the inside of both water-jacketed and direct heat incubators. Many incubators also allow for the CO_2 valve to be adjusted when internal conditions are disturbed, such as opening the incubator door to deliver more cell plates, so that the environment is always stable.

Clinical Significance

There is a significant need for live cells to be cultured via the assistance of an incubator. Pharmaceutical companies often use these methods for drug development and testing as live cell imaging can be used to screen chemicals, cosmetics, and other drug components for their efficacy [8]. Live cell imaging is important because it allows for observation of internal structures and cellular processes in real time. These observations allow for more insight into the process of a cell, rather than viewing snapshots. Pharmaceutical companies can also access the drug cytotoxicity in different cell types. Virology and vaccine products benefit from live cell cultures as it can be used to study viruses in order to make new vaccines, such as in the product of the SARS-COVID19 vaccine [1]. Embryonic stem cells are widely studied for their regeneration properties due to cell cultures and genetic engineering/gene therapy using cultures to study the expression of specific genes and the impact they have on cells in the body.

Client

The client for the Microscopic Cell Culture Incubator is Dr. John Puccinelli, an undergraduate advisor and professor in the Department of Biomedical Engineering at the University of Wisconsin-Madison. The client will be using this product during a teaching lab where students will conduct live cell imaging on tissues for up to a week at a time. The specifics of the experiment are unknown, however it is believed to be used to teach students how to image cells and watch cellular growth over the course of the week. Having a cell culture incubator that is compatible with an inverted microscope will provide easier teaching and preparation methods for professors. Less time will be spent transferring cells from an incubator or disassembling a bulky microscope assembly and more time will be spent developing the main learning objectives of the course.

Product Design Specifications

The client has asked the team to create an incubation chamber that must be able to maintain an internal environment of 37℃ ± 0.5°C, 5% ± 0.1% CO_2, and 95-100% humidity with even heating and humidity across the chamber. Even heating is defined as a consistent temperature throughout each section of the chamber. The incubator must also fit on an inverted microscope stand (roughly 310x300x45mm) without interfering with the microscope's optics and functionality. Therefore, the top and the bottom of the incubator must be transparent in order for imaging through the chamber. The aim for this project is to be able to make a device that is easily assembled/disassembled, disinfected, and can be moved between uses. The market for this product is teaching labs, but if more successful, it could be marketed towards other laboratories and pharmaceutical companies. For more information, see the Full PDS in Appendix A.

III. Preliminary Designs

Design #1 Past Project Refurbished

This is the sixth semester a group has worked on this project for the client, however Figure 1 displays a past project that may work with more alterations and/or improvements [9]. No group has been successful at fabricating a fully functional microscope cell culture incubator. For this reason, continuing to work on this design to further test the product, improve materials, and fix coding errors regarding the sensors was a realistic option. Every previous design involved a rectangular box for the incubation chamber. The design also included a glass top that minimized optical impairment and allowed the incubator to go through sterilizations while extruding less heat loss. The bottom part of the chamber had a transparent heating element. The CO_2 input tube was linked on both sides of the chamber [10]. Lastly, sensors that controlled CO_2, temperature, and humidity were connected to an Arduino microcontroller. The disadvantages of this design were finding quality materials that could keep CO_2 levels and temperatures constant while being within a low-cost budget.

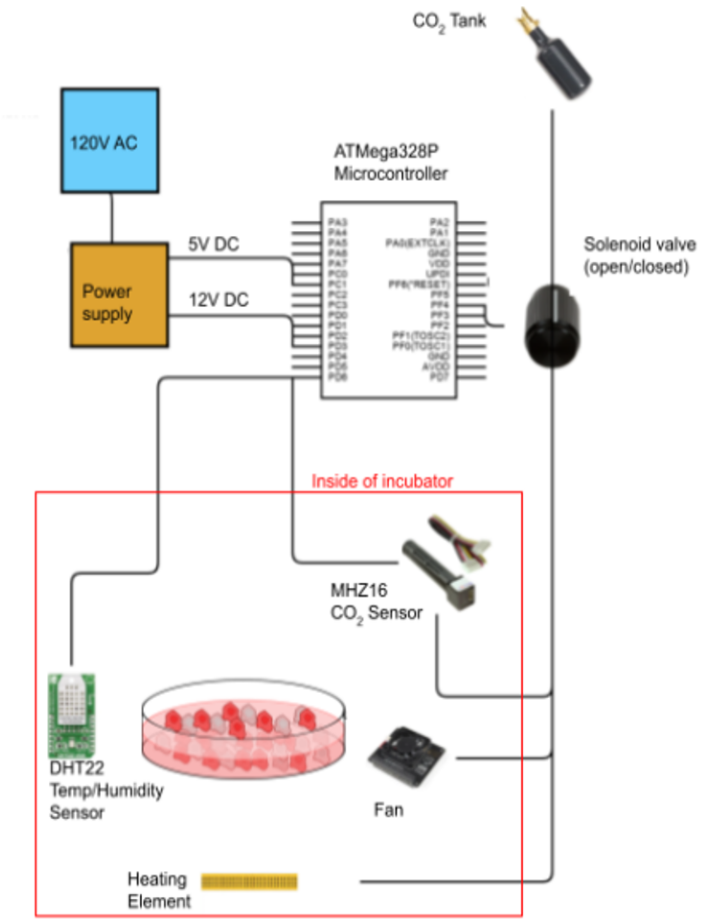


Figure 1: Past BME Design project schematic for incubator design

Design #2 Heated Water Pump Incubator

The heated water pump incubator (Figure 2) will consist of an outer and inner box. The inner box will be where the cell plate is placed and stabilized. There will be transparent glass on the top and bottom of the cell culture incubator to incorporate the inverted microscope. Design #2 received its name based on the heating mechanism used in this incubator. A conducting metal tube will be wrapped around the inside of the incubator and connected to a heated water pump that will be set to 37°C. The inside of the incubator will be filled with water, submerging the metal tube, allowing the internal environment to be heated by conduction as well as increase the humidity to >95%. The incubator box will also include a tube connector to allow CO_2 gas to be pumped in. Lastly, a separate box will be placed inside the incubator to allow for wiring and sensors to be inside the internal environment. The sensors will be connected to an Arduino microcontroller where temperature, humidity, and CO_2 levels will be collected and analyzed.

Item NO.	Item Description	Dimensions (mm)	QTY.
1	Top glass plate	250 x 200 x 5	1
2	Sealed glass plate holder	260 x 210 x 6	1
3	Metal tube for water	d = 7.16	1
4	Outer box of incubator	250 x 200 x 28	1
5	Inner box of incubator to hold cell plate	140 x 96 x 18	1
6	Lower glass plate	250 x 200 x 5	1

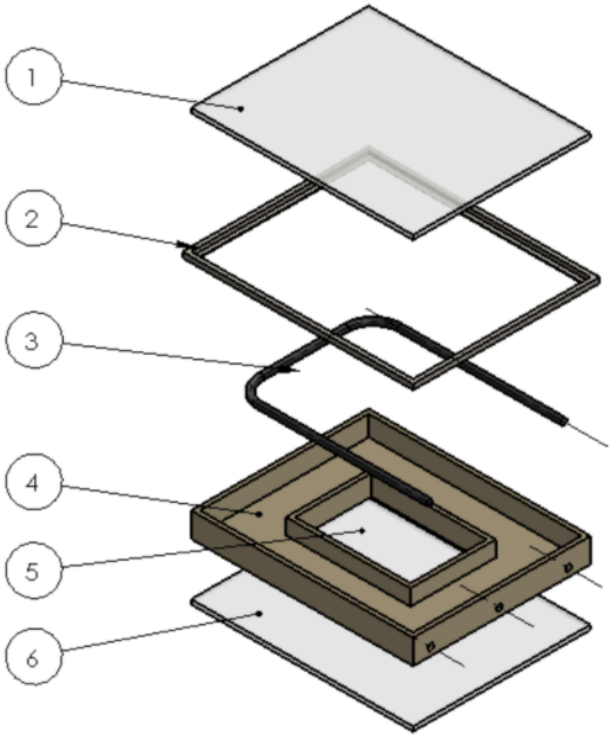


Figure 2: Exploded view of SOLIDWORKS drawing of heated water pump with item descriptions[1]

[1] See Appendix B for SOLIDWORKS Drawing

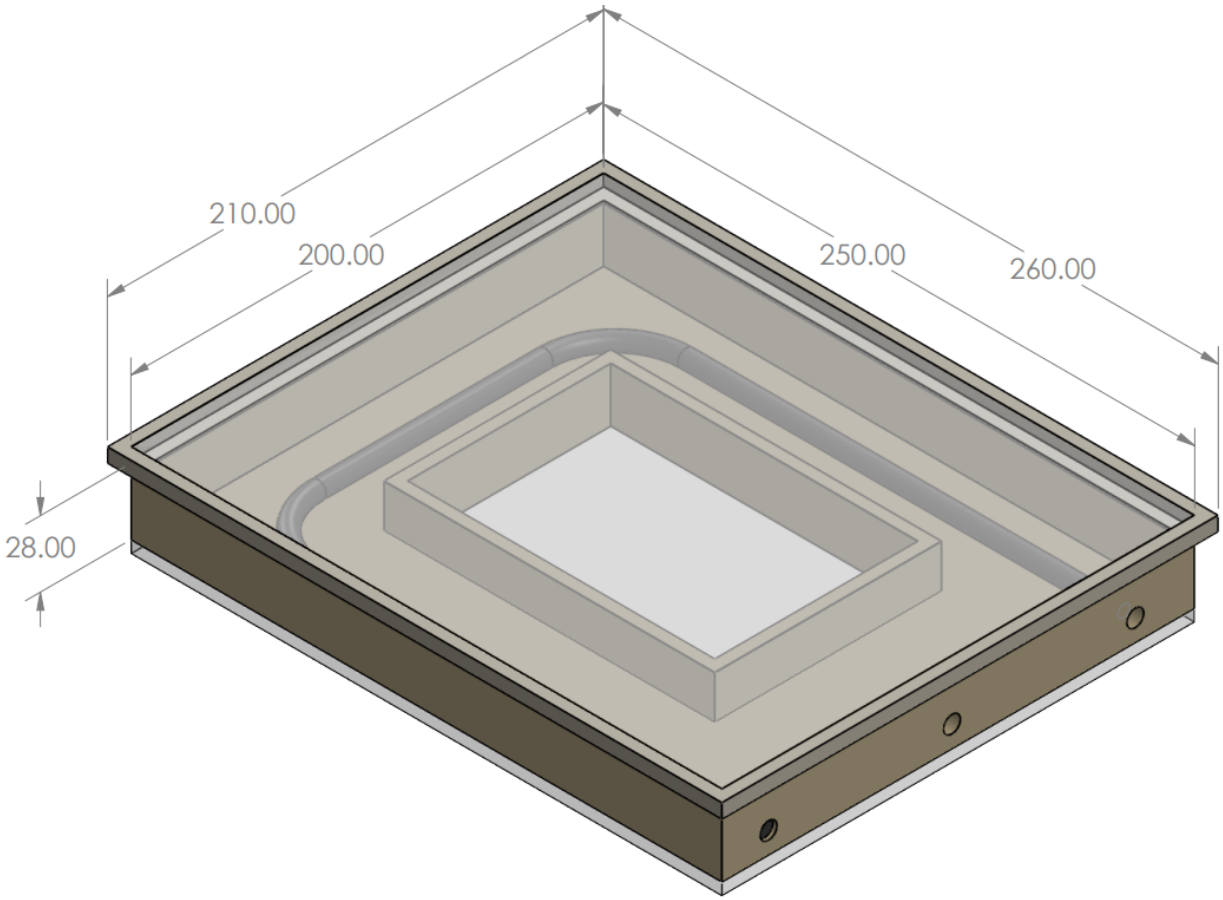


Figure 3: Collapsed view of Heated water pump incubator design with dimensions in mm.

Design #3 Shelving Incubator

The shelving incubator design was created as a multi-shelf system that is able to hold a variety of well plates within it. A small box would be created with a glass panel over the front face of the structure, with a hinge that allows for the opening and closing of the incubator. Each shelf would be sealed with separate incubation pods that allow the user to observe well plates without disrupting the internal environment of the system. Each shelf would also be capable of sliding out of the incubator for further inspection without disrupting the other well plates in the incubator. A track would also be placed on the left and right sides of the incubator in order to vertically move the incubator along the microscope stand for visual inspection of each shelf. The well plate of interest would then be located under the microscope lens for observation. As a whole, this device would succeed in both observation, growth, and protection of multiple well plates for complex research purposes.

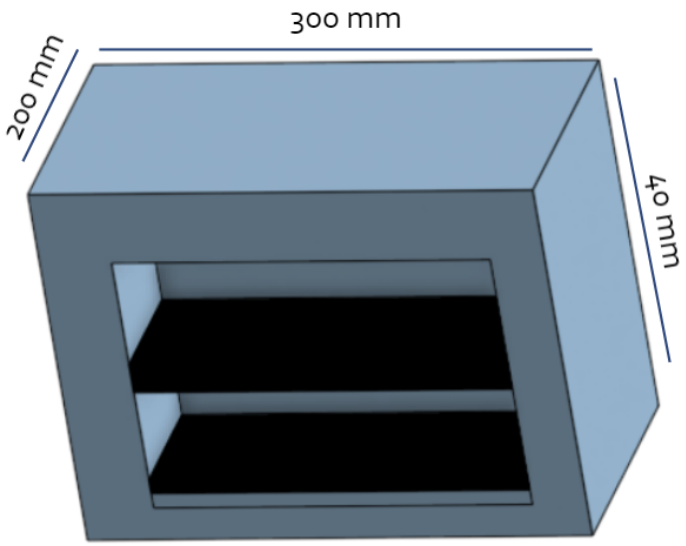
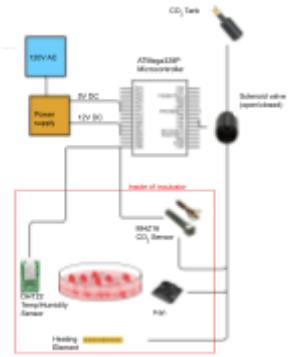
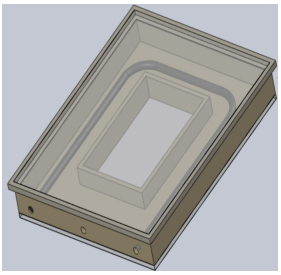
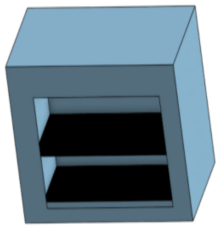


Figure 4: Shelving Incubator Design #3

IV. Preliminary Design Evaluation

Design Matrix

Table 1: Design Matrix with all methods scored on internal environment maintenance, microscope compatibility, accuracy and reliability, ergonomics, cost, life in service, and safety.

		 [11]					
		Past Project Refurbished		**Heated Water Pump Incubator**		**Shelving Incubator**	
Criteria	Weight	Score (10 Max)	Weighted Score	Score (10 Max)	Weighted Score	Score (10 Max)	Weighted Score
Internal Environment	25	9	23	7	18	5	13
Microscope Compatibility	20	10	20	10	20	10	20
Accuracy and Reliability	20	7	14	8	16	4	8
Ergonomics	15	5	8	8	12	4	6
Cost	10	2	2	4	4	3	3
Life in Service	5	10	5	10	5	10	5
Safety	5	10	5	10	5	10	5
Sum	**100**	**Sum**	**77**	**Sum**	**80**	**Sum**	**60**

Scoring Criteria

Internal Environment: The internal environment maintenance was weighted the highest due to the client's request that these standards be met as close to industry standards as possible, with

some leeway provided the internal environment is viable with live cells. Since live cells are being used in the cell cultures, the incubator must be able to meet 37℃ ± 0.5°C, 5% ± 0.1% CO_2, and 95-100% humidity, in order to survive for the duration of the teaching lab.

Microscope Compatibility: Many currently available incubators are not compatible with inverted microscopes as a result of their size and price. The team needed to design an incubator to fit onto an inverted microscope stand, roughly 310x300x45mm. The team's current designs are much smaller than current incubators. The final product must not interfere with the microscope's optics, allowing for transparency for top and bottom viewing of the cells, along with a maximum thickness of 45mm so that the product does not come in contact with the lens of the scope.

Accuracy and Reliability: Due to the importance of the internal environment for cell growth, the incubator must be able to regulate the conditions within a small margin of error. The accuracy and reliability of the device will be evaluated and monitored using temperature, humidity, and CO_2 sensors connected to the device via an Arduino microcontroller.

Ergonomics: The device must be within a size and weight that the average user can safely handle and move with ease.

Cost: The total cost of the product has a budget of $100, although the client has said that more funds may be provided based on the success of the initial prototype.

Life in Service: The final product will need to be used for one week out of the semester in the client's teaching lab. The shelf life of this product has a minimum of 10 years.

Safety: The product needs to adhere to FDA and OSHA standards and regulations [12][13]. Due to the use of tissue cells, the incubator must abide by Biohazard Safety Level 2 and ISO Class 5 air quality standards [14][15].

Proposed Final Design

The team chose to move forward with the second design, The Heated Water Pump Incubator. This design fits the clients needs the best because it will produce the most accurate and reliable internal environment. The use of a heated water pump containing the desired temperature of 37°C throughout a smaller space not only ensures homogeneous temperature, but also helps maintain humidity as it is compatible with a low volume water bed that can be placed in the incubator. The design is also relatively small allowing for easy assembly, ease of use, and can be readily disassembled and interchanged for another type of cell culture. The design is compatible with the microscope currently being used by the client, and also other microscopes in the teaching lab as it is wider in length than it is in height allowing for it to be used with smaller

microscopes. The Heated Water Pump Design was also the lowest in cost, with materials from past semesters being used to ensure that the target budget is not exceeded. This design was lowest in cost as opposed to the past projects because previous works had a much larger budget than is currently allotted. Design #2 received the second highest score for internal environment because Design #1 would allow for the internal environment to be most easily reached due to the simplicity of heating elements compared to a more complex water and tubing design. All of the designs were deemed microscopically compatible, safe to use, and have comparable shelf lives, resulting in equal scores. Overall, the Heated Water Pump Incubator won over the other two due to its compactness, accuracy and reliability, and low production cost.

V. Fabrication/Development Process

Materials[2]

Arduino Materials

The device will be made with an Arduino sensing unit for the purpose of measuring temperature, humidity, and CO_2 levels during incubator usage. A DHT22 sensor was previously used in past projects as it accurately and reliably measured both temperature and humidity. However, the downside to this material is that it is not waterproof. The team opted for a thermistor, which measures temperature and is waterproof. The thermistor is also smaller allowing for better implementation into the incubator. In order to make sure that the thermistor can read both temperature and humidity, the team used an equation (see Appendix D) to determine the relative humidity inside the incubator. The accuracy of this equation was tested against the DHT22 temperature and humidity sensor.

In order to measure CO_2 levels inside the incubator, the team used a MH-Z16 NDIR CO_2 sensor, which has been used in past projects. This material was chosen because it is waterproof, has the ability to read temperature which would allow homogeneity of heat throughout the incubator to be checked, and because it was already available for use which would help the team stay under budget.

Incubator Materials

The incubator part of the cell culture will be made using white PLA plastic, 3D printed at the University of Wisconsin Makerspace. PLA plastic was the main material used for the incubator box because of insulation properties, accessibility, and overall cost per gram of the material. Inside the box, vinyl tubing was chosen to supply hot water to the incubator in order to create the necessary temperature and humidity. The team chose vinyl because it is low in cost, waterproof, ductile, and is able to transfer the necessary amount of heat while also withstanding the temperature of water running through it. The waterproof capabilities of the tubing are needed

[2] See Appendix F for Materials and Expenses

because it will be submerged in a low volume bed of water in order to create humidity and provide a homogenous temperature inside the incubator. The ductility of the vinyl is also beneficial as it will be wrapped twice around the inside of the box in order to increase surface area and allow for heat to be spread evenly and more efficiently throughout the incubator. A vinyl tube with an inside diameter of ¼ inch and an outside diameter of ⅜ inch was chosen for the final prototype. In order to connect the vinyl tubing to the water supply, a nylon barbed vacuum connector ⅜ x ⅜ inch was chosen. The barbed connector was chosen as it helps provide leakproof connections, allows for easy, push-fit installation, and is ideal for connecting vinyl hoses together.

Methods

Thermistor

The thermistor sensor was coded (see Appendix D) to measure both the temperature and the relative humidity inside the incubator. The relative humidity was measured using Magnus' form approximation of saturation vapor pressure, (Equation 1) where T is the temperature measured and RH is the relative humidity[16][17]. The accuracy of this equation was later tested against a DHT22 temperature and humidity sensor. The equation was adequate in measuring relative humidity as the substance producing the humidity inside the box was water and the dew point of water was known as 5.2℃.

$$RH = 100 * \left(\frac{1.4466}{exp\left(\frac{17.625T}{243.04+T}\right)}\right) \quad [\%] \ (1)$$

The thermistor was connected to an Arduino microcontroller and then separately inserted into the bottom right corner of the incubator box. It was placed here for the purpose of measuring the internal temperature away from the source of the heat, so that the data was not skewed.

CO_2 Sensor

The MH-Z16 NDIR CO_2 sensor was coded (see Appendix E) to measure both the concentration of CO_2 inside the incubator as well as the percentage of CO_2. The sensor was connected to a separate Arduino microcontroller and placed into a corner of the incubator box. The sensor was connected to a separate Arduino microcontroller so that the user could view the Serial Plotter for both the temperature/humidity and the CO_2 concentration/percentage and export each data set separately. This method also made it easier for the team to conduct testing as each individual sensor could be looked at without interfering with the other. The placement of the CO_2 sensor was away from the source of the CO_2 gas to ensure that data was not skewed and that the internal environment was stable at all points in the incubator.

Final Prototype

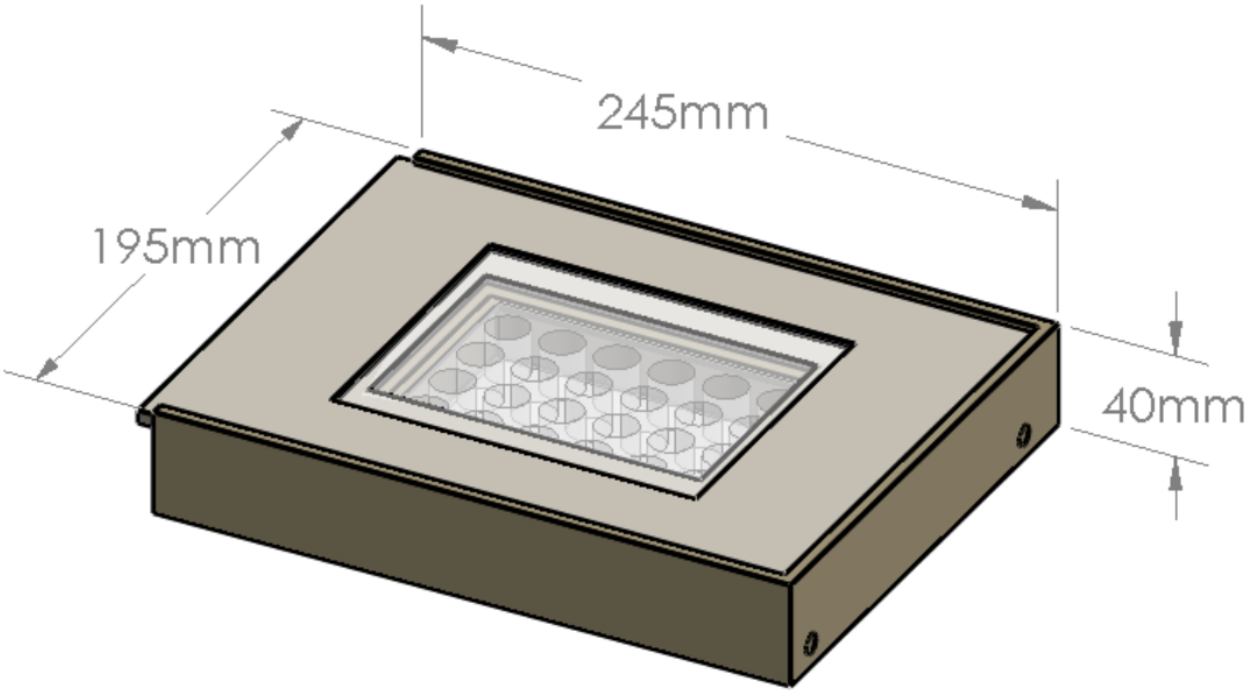


Figure 5: Final Prototype SOLIDWORKS Drawing[3]

The final design consisted of a 195x245x40mm 3D printed PLA box, with two roughly ⅜ diameter holes, drilled with ⅜ drill bit but expanded using a circular file, in order for ⅜ inch barbed vacuum connectors to be inserted. The two sheets of frosted polycarbonate glass were then hot glued to the corresponding flatbeds on the incubator. Five feet of vinyl tubing (⅜ x ¼ inch) was wrapped in a circular motion around the inside of the box and connected to the vacuum connectors. These tubes were secured into place via zip ties. Vinyl tubing (½ x ⅜ inch) was then secured to the valves on the hot water pump and zip tied for security. The incubator also had a ½ inch diameter hole drilled into the bottom right corner of the incubator and expanded with a circular file in order for the CO_2 sensor to enter the incubator. The CO_2 sensor was then fitted and hot glued into this opening. The thermistor was placed in the incubator by drilling a ¼ inch diameter hole into the bottom left corner and hot gluing the thermistor into place. Finally, roughly 16oz of water was added to the interior of the incubator in order for the heat from the water pump to be conducted, heating up the incubator at a faster rate and allowing for even heating and humidity throughout the incubator.

[3] See Appendix C for SOLIDWORKS drawing of final design

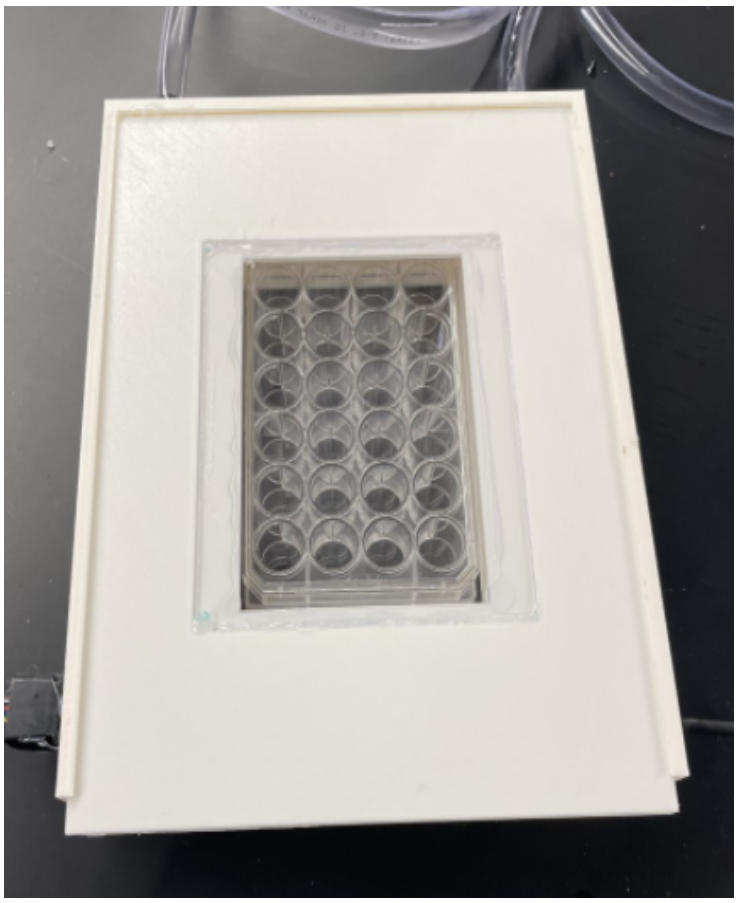


Figure 6: External View of Incubator

Figure 7: Internal View of Incubator

Testing

The team will be testing the accuracy of the proposed design in the client's cell culture lab in order to determine if the internal environment is stable and if the microscope optics are not corrupted. (See Appendix G for Testing Protocols)

Temperature Testing

The ability for the thermistor to accurately record whether the incubator maintains an internal temperature of 37°C ± 0.5°C was evaluated using the Internal Environment - Temperature and Humidity Sensor Testing Protocol[4]. First, the sensor was calibrated using resistance values given by the Arduino website. Once the sensor was calibrated, its precision in a dynamic range was evaluated by first measuring the temperature and humidity of the working environment to gauge if they are both working as expected, and then measuring its temperature at extreme high and low temperatures using a hair dryer and freezer.

Next, the accuracy of the thermistor was evaluated by placing it into the lab incubator and ensuring it reads the temperature the incubator is set to within an error range of ± 2°C. After

[4] See Appendix G for Testing Protocols

placing the sensor in the lab incubator for 10 minutes, the temperature reading was ensured to accurately record the incubator temperature over the entire time interval.

Finally, the temperature sensor was tested within the Microscope Cell Culture Incubator itself. The incubator was set up for normal use, and the sensor and a digital thermometer were placed within the incubator before it was sealed. The ability for the incubator to maintain a temperature of 37°C ± 0.5°C was tested by taking measurements every 10 seconds over a period of 10 minutes and verifying it stays within the optimal range. Then, the ability for the sensor to accurately measure the temperature within the optimal range was evaluated by taking measurements every 10 seconds over a period of 10 minutes and verifying the thermistor records temperature values of 37°C ± 0.5°C.

If all these tests were passed, the thermistor and the incubator's ability to maintain the temperature internal conditions were approved. If any of these tests were not verified, then the incubator was reassessed at that point before approval.

CO_2 Testing

The ability for the CO_2 sensor to accurately record whether the incubator maintains an internal environment of 5% ± 0.1% was evaluated using the Internal Environment - CO_2 Sensor and Feedback System Testing Protocol[5]. Once the sensor was calibrated, its precision in a dynamic range was evaluated by ensuring its values increase and decrease with general increase and decrease of CO_2 concentration. The sensor was first tested in room conditions to ensure it gave a consistent reading. Then, the sensor was exposed to an increased concentration of CO_2 by having group members breath on the sensor and the sensor readings were observed to ensure it increased in value. Similarly, the CO_2 supply was cut off and a decrease in concentration readings from the sensor was verified. If the sensor increased and decreased in CO_2 percentage readings as expected, then its precision in a dynamic range was approved.

Next, the accuracy of the CO_2 sensor was evaluated by placing it into the lab incubator and ensuring it reads the concentration the incubator is set to within an error range of ± 0.1%. After placing the sensor in the lab incubator for 10 minutes, the CO_2 sensor reading was ensured to accurately record the incubator temperature over the entire time interval.

Finally, the CO_2 sensor was tested within the Microscope Cell Culture Incubator itself. The incubator was set up for normal use, and the sensor and a fyrite were placed within the incubator before it was sealed. The ability for the incubator to maintain a concentration of 5% CO_2 ± 0.1% was tested by taking measurements every 10 seconds over a period of 10 minutes and verifying it stays within the optimal range. Then, the ability for the sensor to accurately measure the CO_2 concentration within the optimal range was evaluated by taking measurements every 10 seconds over a period of 10 minutes and verifying the sensor records concentration values of 5% CO_2 ± 0.1%.

[5] See Appendix G for testing protocols

If all these tests were passed, the CO2 sensor and the incubator's ability to maintain the CO_2 internal conditions were approved. If any of these tests were not verified, then the incubator was reassessed at that point before approval.

Optical Testing

The optical clarity of the Transparent Polycarbonate sheets were evaluated qualitatively and quantitatively to ensure they do not impair the microscope's ability to view the cell culture. First, the sheets were evaluated qualitatively. The microscope and its imaging software were prepared for use. Then, one team member placed a prepared slide under a sheet of the High Transparent Lexan Polycarbonate and placed those two onto the microscope stage. The microscope was then adjusted to best clarity and an image of what was observed under the microscope was captured. The same procedure was then followed but without the Polycarbonate sheet. To ensure the images quality could be evaluated in a blind and objective fashion, the tester labeled the images and created a key for the naming process. Finally, three team members who were not present for the imaging process assessed the clarity of the two images. Each member chose which image they believe is clearer, or if they looked the same. If the majority could see a difference in clarity between the two images, the test has failed and a different transparent material should be tested for use. If the majority could not see a difference in clarity between the two images, then the Polycarbonate sheets passed the qualitative test.

In the next testing protocol, the clarity of the Transparent Polycarbonate sheets were evaluated quantitatively. The microscope and its imaging software were prepared for use, and then the same imaging process from before was used to acquire two images of the prepared slide: one with the Polycarbonate sheet and one without. Using ImageJ analysis, the clarity of the images using the microscope focus quality plugin was recorded; the images were divided into squares and each square was assigned a color based on their focus level. The assessments of each image were then compared to evaluate their similarities in clarity. If the majority of the regions in both images were the same, then the Polycarbonate sheets passed the quantitative test and were approved for use in the incubator.

Recovery Testing

The ability of the incubator to return to its internal environment of 37℃, 5% CO_2, and 95-100% humidity after a 30 second opening will be evaluated to ensure it returns to these conditions in an efficient manner. The completed incubator will be set up for normal use, and the internal conditions will be recorded to verify they fall within the correct ranges. Once the ability for the incubator to maintain the internal conditions is confirmed, the data collection from each sensor will begin. The incubator will then be opened for 30 seconds, and it will be ensured each sensor records a deviation from the internal conditions. Then, the incubator will be closed and a stopwatch will start while conditions are monitored to see if they return to normal. Once temperature, humidity, and CO_2 individually return to their respective mark for optimal internal

conditions, the time from when the incubator was closed will be recorded. If a condition does not return to its range after 15 minutes, this will be recorded. If every condition returns to 37℃, 5% CO_2, or 95-100% humidity within 10 minutes after the opening, then the recovery of the incubator is approved. If one of the conditions does not return to its mark, then that condition needs to be reevaluated and the recovery testing will occur again.

VI. Results

Temperature Testing Results

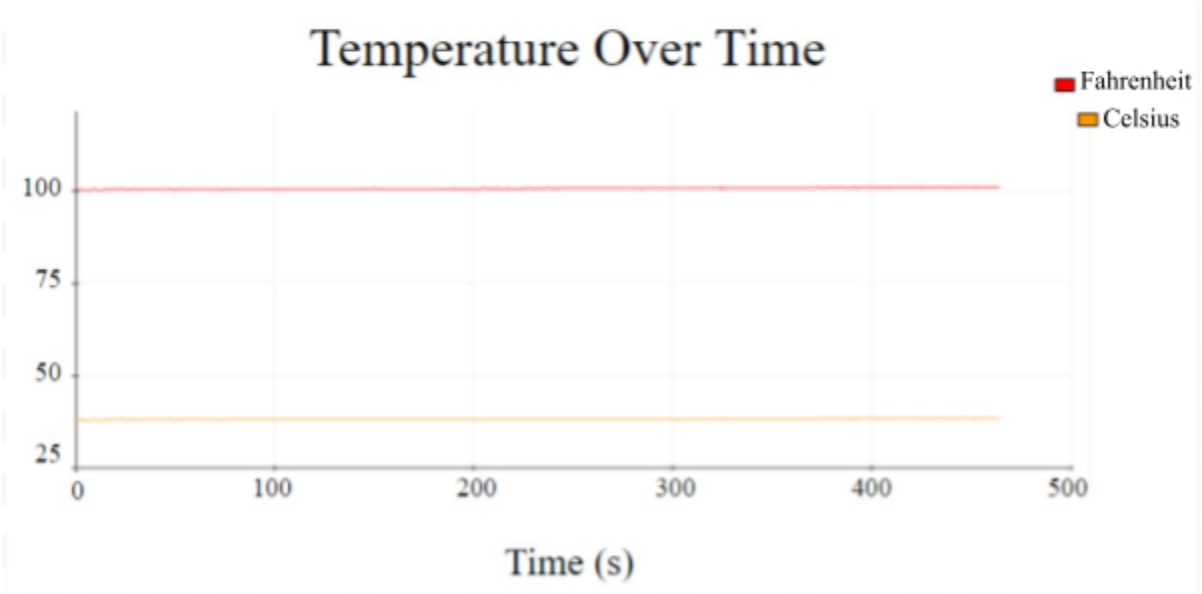


Figure 8: Thermistor Reading Over 10 Minute Time Interval

The team analyzed the data from the testing done using the thermistor to determine the stability of it's readings over the course of a ten minute time interval. The thermistor output the temperature in both Fahrenheit and degrees Celsius, both of which corresponded to the known value being reported on the incubator already being used in the teaching lab. The results show that the thermistor sensor is correctly analyzing the internal environment of the incubator.

Humidity Results

Table 2: t-Test for Two Samples Assuming Unequal Variances

t-Test: Two-Sample Assuming Unequal Variances

	DHT22	*Thermistor*
Mean	12.61830986%	12.16718182%
Variance	0.090374245%	0.424219419%
Observations	71	220
Hypothesized Mean Difference	0	
df	255	
t Stat	7.973463829	
P(T<=t) one-tail	2.59912E-14	
t Critical one-tail	1.650851092	
P(T<=t) two-tail	5.19824E-14	
t Critical two-tail	1.96931057	

The team decided to use Equation 1 to code the thermistor to output humidity as well as temperature values. Since the team was also in possession of a DHT22 Temperature and Humidity sensor, the DHT22 sensor was used to measure the accuracy of the formula. In a room temperature environment, the thermistor and the DHT22 sensors ran for approximately 10 minutes. The humidity data collected from both sensors were then put to a two sample t-Test with a significance level of .05, where the humidity values for the thermistor were being compared to the humidity values of the DHT22. The DHT22's mean and standard deviation were 12.618% and 0.30%, while the thermistor's mean and standard deviation were 12.167% and 0.651%. The results showed that the values outputted from the thermistor were statistically significant, meaning the team is 95% confident that the means of each data set were not similar, even though the averages both fell within the team's desired output and error range. This test may have been statistically significant because of the large sample of numbers and precise quantitative data being produced by the sensors. In the future, the formula being used should either be revised, or a new approach to calculate humidity is needed in order to produce a two sample t-test that does not have statistically significant data.

CO_2 Testing Results

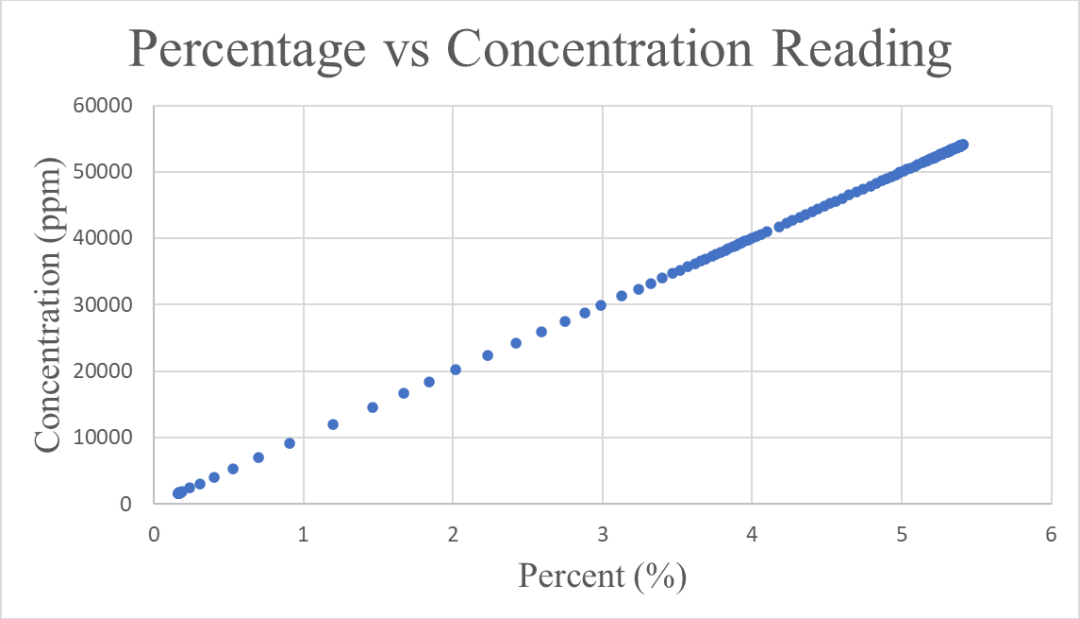


Figure 9: CO_2 Percentage Reading vs Concentration

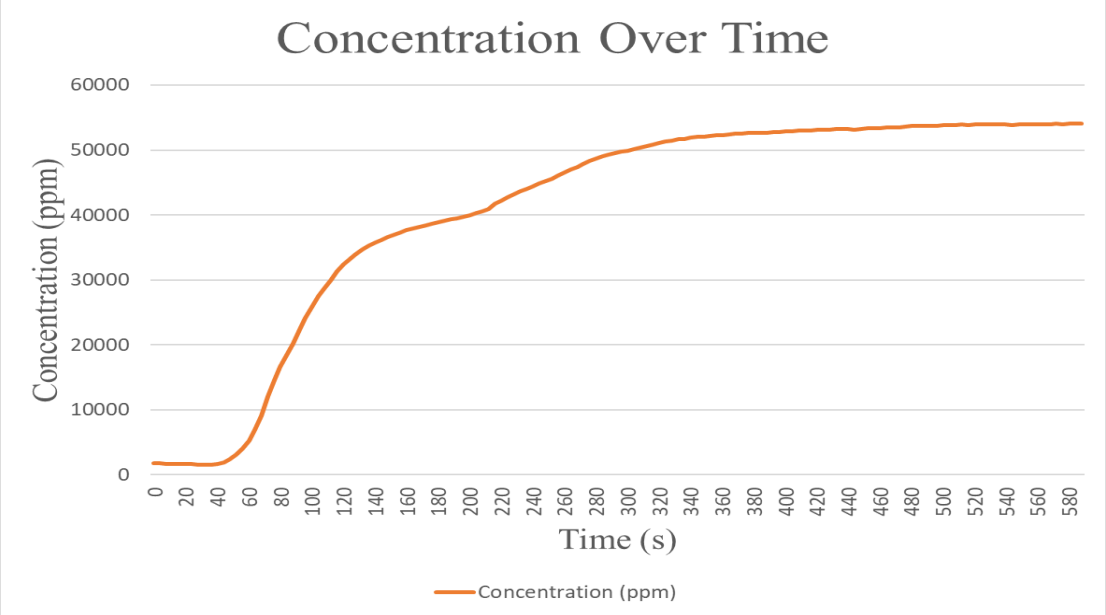


Figure 10: CO_2 Concentration Over Time

The results of the CO_2 testing were obtained via measuring the concentration and computing the percentage of CO_2 in the teaching lab's incubator to determine the accuracy of the sensor. The results prove that the percentage of CO_2 corresponds to the correct concentration, and is equal to that of the incubator with an error of $\pm$ 0.1%. The CO_2 sensor was also placed from a room environment to an incubator and the time it took to stabilize was assessed. The

results show that the sensor is able to read the correct internal environment concentration after roughly 5 minutes of incubation. The concentration of CO_2 is then steady for the remainder of the data collection, proving that it will be sufficient at measuring the concentration and percentage over the week-long course of use in the client's lab.

Optical Testing Results (Prior and After Installation)

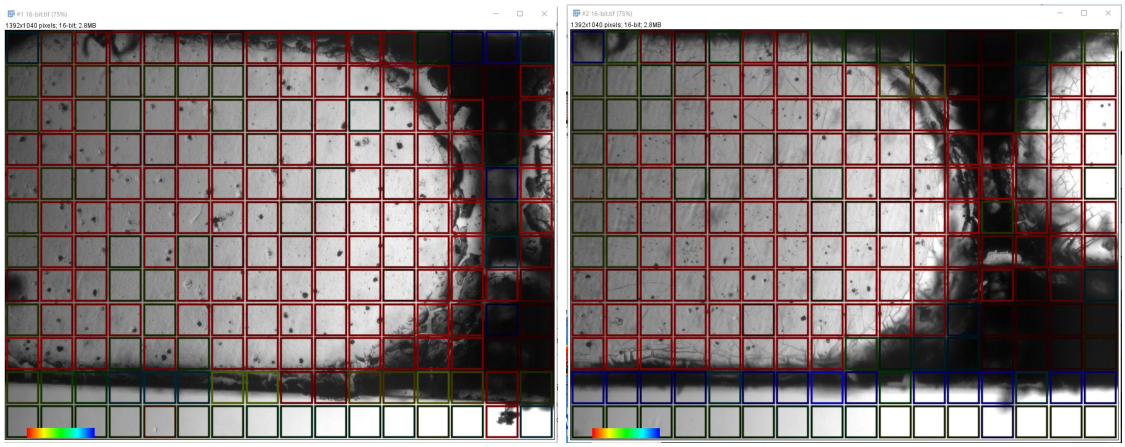


Figure 11: Optical analysis from ImageJ of microscopic cells with glass (left) and without glass (right)

Table 3: Table displaying the number of red (in focus), green (mid focus), and blue (out of focus) squares shown in each image above

	Microscope Image with Glass	**Microscope Image without Glass**
Red Squares	130	120
Green Squares	54	51
Blue Squares	8	21
Total	192	192

The two optical testing images above show boxes around the image that outline the clarity and quality of that part of the image. According to the color scale shown at the bottom of both images, the red end of the spectrum indicates that the image is in focus at a specific region, while the blue end of the spectrum indicates that the image is out of focus at a given region. Results from this test show that the image with the glass had a slightly higher, yet very similar focus quality compared to the image without glass present. Similarly, 100% of randomly selected subjects expressed no difference in clarity between the two optical images. As seen above, the microscope image with glass has slightly more red squares (in focus) and fewer blue squares (out of focus), causing it to have a higher focus quality. However, the two images have very similar values for each color type as demonstrated in Table 3. It is important to note that these two

images are slightly different because the image with the glass is more zoomed in on a cell than the image without the glass. This may have contributed to a slightly higher focus quality for the image with the glass rather than without the glass. In the future, this test can be improved by identifying the same cell with and without the glass, to ensure a higher accuracy of the results.

Recovery Testing Results

The recovery testing protocol was not able to be executed as the CO_2 tank was not available for use for the team this semester. Recovery testing was also not possible due to the inability of the box to reach the desired internal conditions, and because there was a slight leak in the box during testing.

Final Prototype Results

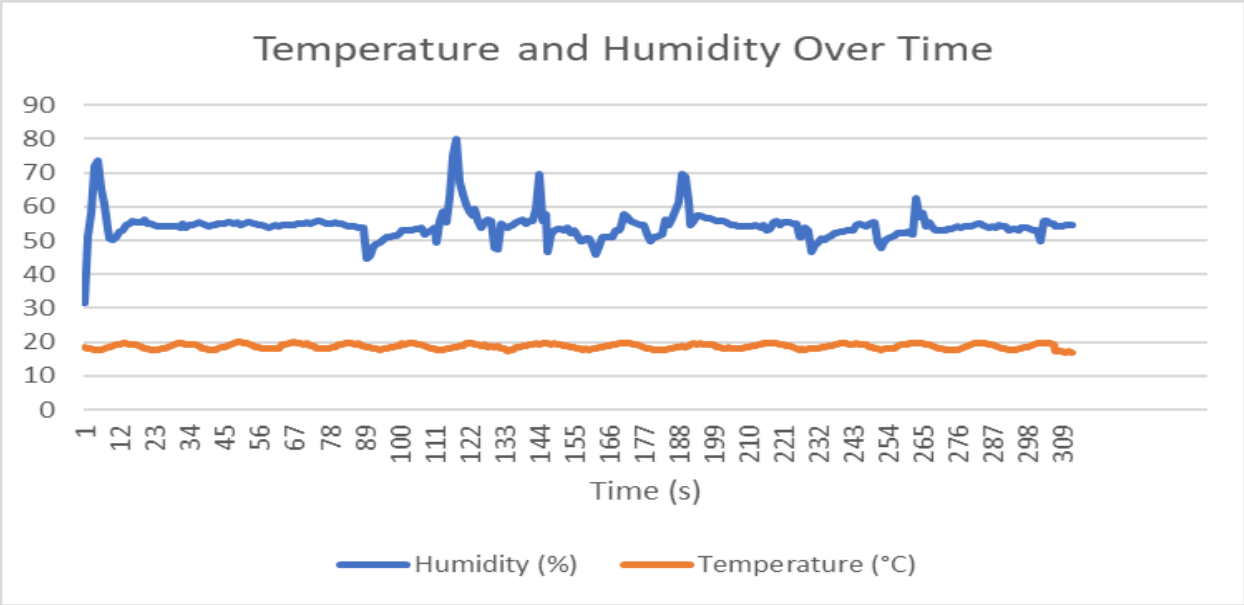


Figure 12: Temperature and Humidity in Incubator Over Time

The results showed that over a ten minute period, the vinyl tubing did not allow for enough heat transfer to reach an optimal temperature of 37℃ ± 0.5°C, however the thermistor reading was consistent over the course of testing. This proves that the thermistor is able to accurately measure the internal temperature of the incubator. The humidity analyzed was also not able to reach a minimum of 95% humidity, although the formula used to calculate the relative humidity inside the incubator must be revised in order to have accurate and reliable results. The PLA plastic used to make the box also caused issues, as it is a fairly brittle material and the plastic left small gaps where water would leak out during testing. The result of this is that PLA plastic should not be used in the future with this device.

VII. Discussion

In conclusion, the team has been able to fabricate an incubator with precise sensors, transparent visuals, and a working water-pump system, however the material used for the box causes leakage, the vinyl tubing does not allow for adequate heat transfer, and the CO_2 aspect of the design was not able to be incorporated due to a lack of CO_2 tank availability. The thermistor was able to accurately read the temperature in both Celcius and Farenheit, while also outputting relatively accurate humidity data. The t-test conducted for the humidity formula used in the code for the thermistor as compared to the values produced by the DHT22 sensor did state that the values were statistically significant ($p < 0.05$). However, the means of the two measurements over a five minute interval had a deviation of ±0.5°C. The CO_2 sensor was tested in the available incubator in the teaching lab in 1002 Engineering Centers Building and it was concluded that the sensor was able to output the correct percentage of CO_2 in the tank as compared to the value presented on the screen of the incubator. The incubator was able to fit underneath the microscope and water was able to run throughout the tubing in the interior of the box. The water was able to heat up via the heated water pump and the sensors were able to read a temperature and humidity difference at different water temperatures. There was leakage in the box after approximately 30 minutes of usage, however it was undetermined where the leak originated from. After 30 minutes of usage, the water bath was still not able to reach the desired temperature of 37℃ and the amount of humidity had caused the glass to become foggy. Overall, the materials chosen for this project must be revisited in order to reach the specifications outlined in the PDS and for the optimal success of the incubator as a whole for use in a teaching lab.

As a result of the experimentation, it has been determined that there are three main areas of the project that need revision in the future. The first revision needed to be made is that the relative humidity formula must be updated in order for the thermistor to produce accurate and reliable measurements. More research must be conducted in order to figure out the best way to measure humidity in the incubator and that method must be tested against the DHT22 sensor. The value between the thermistor's humidity output and the DHT22 output should not be statistically significant in the future. The second revision to the incubator must be the type of conductive tubing used inside the incubator box. The vinyl tubing proved to be a better insulator than a conductor, therefore a material such as metal should be used in the future in order to quickly, efficiently, and accurately warm up the water bath in order to reach a temperature of 37 °C± 0.5°C. More research is needed to determine the effects of having a metal submerged in water for a week at a time, in order to ensure that the product still meets the necessary shelf life requirements of ten years. The final revision needed to be made is the material and fabrication process of the incubator box itself. The results show that PLA plastic is not successful for use in this product. After consulting with the client and MakerSpace employees, the team also determined that choosing a thicker, more insulating material and laser cutting it, as opposed to 3D printing would allow for more heat to be contained in the box, as well as lowering production costs. This is due to the fact that laser cutting is a cheaper alternative to 3D printing. Further consideration should also be given to finding better ways to seal the incubation box, such as

using a rubber lining. In the future, the team would also like access to a CO_2 tank in order to both test the sensor and the new fabricated incubator to determine whether or not this aspect of the design is working as expected.

Some sources of error during this experiment, might be that the temperature and humidity readings were not as accurate due to the fact that the crown of the incubator, or the lid, was slightly larger in length than the incubator. This allowed for the user to easily slide the top on and off of the device, but may have resulted in a less insulated structure, prone to loose seals. The temperature inside the incubator may have been lower than expected due to this. The formula used to calculate the relative humidity inside the incubator was also statistically significant to the control sensor used. The collected humidity data is therefore not accurate and is a source of error in the project. Further work needs to be conducted in order to obtain correct measurements. Other sources of error may include that the vinyl tubing used for the water pump may not have been secured tightly, as they were only kept in place via zip ties, and that the tubing connecting the incubator and heated water pump may have been too long, leading to a loss of heat before reaching the incubator. Lastly, fingerprint marks could have interfered with the optics of the glass panels despite efforts to clean the glass regularly.

Ethical considerations need to be taken into account as this device will be used in a live cell lab. The origin of the cells being studied is of the utmost importance. The client plans to use immortalized pre-osteoblasts isolated from the calvaria of newborn mice. The use of animal cells has caused much ethical controversy over the past half-century. Mice are commonly used in laboratory research as their entire genome has been sequenced and compared to the human genome and they are easily bred and housed [18]. Extra measures must be taken to ensure that the newborn mice are subject to the least amount of harm, distress, and pain in order to conduct an ethical experiment. The Animal Welfare Act, a federal law that outlines the standard of care animals must receive in laboratories, is also a necessary requirement of labs to follow when using mice, and other AWA approved animals, with the incubator [19]. If, in the future, human cells are used, the consent of the subject must be granted before cells are placed in the incubator. Ethical consideration must also be given if the cells are to be manipulated in the future, rather than just watching the growth of the cell. Gene editing has become quite the controversy over the past 20 years, with the ethical considerations of its use in treating cancer, preventing life-threatening diseases in gestation, and its use in what has been termed “designer babies:” the idea that one can alter the DNA in a prenatal cell to fit the desired phenotype or genotype of the parents. Designer babies are currently legal in Sweden, Spain, Belgium, the UK, and the US [20]. Furthermore, ethical considerations must be made when determining how manipulations of the cell will alter not only the DNA, but evolution as a whole. The societal implications of prescribed DNA mutations must also be taken into account as the effects of this process can range from the elimination of genetic diseases to the elimination of certain phenotypes altogether.

VIII. Conclusion

The client is in search of a microscopic cell culture incubator compatible with an inverted microscope that is lightweight, maintains a stable internal environment, and is cost-effective for the purpose of using it in a teaching lab during the semester. The team has proposed, fabricated, and tested a design that includes a heated water and CO_2 pump in order to increase temperature, humidity, and CO_2 levels. Succeeding the incubator testing, the team concluded that a more conducting material should be used for the inner tubing because there was only a moderate increase in temperature within the box, never reaching the desired number of 37°C. A more insulating and durable material should be used for the incubator box and lid as well because the team experienced leakage from both the water bed and internal environment. Lastly, the sensor coding should be revised to be even more accurate and precise in order to not have statistically significant data between the control sensor and the sensor used in the incubator. Following the optical testing, the team decided the glass used for the incubator passed all testing protocols and would continue to be used for future prototypes. In the future, the overall concepts and designs of the incubator box will be continued with the majority of improvements being within the incubator materials, the sensor's code, and the input of CO_2.

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X. Appendix

Appendix A: Product Design Specifications (PDS)

Function: Develop a low cost cell culture incubation chamber with interchangeable culture plates that is compatible with an inverted microscope and capable of live cell imaging.

Client requirements:

- Incubation chamber must be able to maintain an internal environment of 37℃, 5% CO_2, and 95-100% humidity
- Microscope's optics and functionality must not be damaged
- Maintain even heating and humidity across the chamber
- Create device that stays within a budget of $100
- Ensure that the device can be easily assembled and removed between uses

Design requirements:

1. Physical and Operational Characteristics

a. ***Performance requirements:*** The device must be able to sit on a microscope stand, be transparent on the top and bottom to allow for optical visualization with an inverted microscope, and maintain an internal environment of 37℃, 5% CO_2, and 95-100% humidity.

b. ***Safety:*** The incubator and the cell culture environment must be in corporation with BioSafety Level 1 Standards [1]. Any material and electrical or mechanical machinery must be sterilizable and waterproof.

c. ***Accuracy and Reliability:*** The device must be able to maintain a temperature of 37°C ± 0.05°C throughout the entire internal environment. The humidity must be kept above 95% humidity. CO_2 levels must be 5% ± 0.1%. The incubator must be able to maintain these conditions for extended periods of time and be able to reach these conditions after the incubator has been opened and exposed to the external environment in an efficient manner.

d. ***Life in Service:*** The device must be able to be used for two weeks, but optimal usage will occur for one week at a time for teaching purposes in the client's tissue lab.

e. ***Shelf Life:*** The shelf life of this product should be ten years.

f. ***Operating Environment:*** The operating environment is a clean room. The incubation chamber must be able to maintain an internal environment of 37°C, 5% CO_2, and 95-100% humidity over a long duration of time, without compromising the integrity of the microscope's optics or functionality. Even heating and humidity across the chamber must be maintained to ensure that evaporation does not occur.

g. ***Ergonomics:*** The device should be portable in that one should be able to carry and store the device easily. Wires should not be hanging freely out of the device, and it should be easy to pick up and put away when needed.
h. ***Size:*** The size constraints for this device are that it must sit on the microscope stage and hold a well plate that also doesn't interfere with the optics or functionality of the microscope. It would be ideal if all sides are transparent, but it is a requirement that the bottom and top are transparent. Overall, the product must be compatible with an inverted microscope.
i. ***Weight:*** There are no specific weight requirements. However, minimizing weight would be ideal to promote incubator mobility and usability.
j. ***Materials:*** There are no specific materials that are required for development of this device. However, it is important to examine different material properties to determine which materials hold heat effectively and have a transparent appearance.
k. ***Aesthetics, Appearance, and Finish:*** The client does not have a preference in color. Well plates are clear, black (to stop contamination), and white (to increase light). Using materials that would block out external light sources would be ideal, but this is not a requirement for the device. Finish should exclude messy elements, such as long wires, and be transparent on both the top and bottom.

2. Production Characteristics:

a. ***Quantity:*** Only one device is necessary to produce, but ideally, it would have the capacity to be produced on a larger scale to be used repeatedly in the teaching labs.
b. ***Target Product Cost:*** The target product cost for this device is $100. It will be paid for via UW BME Departmental teaching funds.

3. Miscellaneous

a. ***Standards and Specifications:*** The incubator would need to adhere to the ISO 13485 regulation which outlines requirements for regulatory purposes of Medical Devices [2]. The incubator would also need to follow the FDA's Code of Federal Regulations Title 21, Volume 8 where it outlines the requirements for Cell and Tissue Culture products [3].
b. ***Customer:*** The client, Dr. John Puccinelli, is an undergraduate advisor in the Biomedical Engineering Department at the University of Wisconsin - Madison. Dr. Puccinelli is asking for the cell culture incubator in order to amplify the teaching curriculum in his classroom environment. Having an incubator that is easy to disassemble and compatible with an inverted microscope would result in efficient classroom lessons.

c. ***Patient-related concerns:*** The accuracy of the temperature, humidity, and CO_2 concentration is of utmost concern for the client. Humidity must be 95-100%, otherwise cells will begin to dry out. Having a set temperature of 37°C will replicate optimal cellular environments. Lastly, ease of disassembly and disinfecting of the incubator was of concern.
d. ***Competition:*** There are currently multiple inverted microscopes and cell culture incubators on the market ranging from $500-$40,000 [4]. Thermo Fisher, NuAire, and New Brunswick all have incubators currently on the market. Thermo Fisher and NuAire are more popular as they have both direct heat and water jacketed incubators. The most popular Thermo Fisher design is the Heracell VIOS 160i CO2 Incubator with Copper Interior Chambers, which has HEPA filtration for ISO Class 5 air quality and an overnight Steri-Run for total sterilization [5]. Others have also attempted to design low-cost live-cell imaging platforms using 3D printed and off the shelf components. A team of researchers from Australia were able to successfully design a portable low-cost long-term live-cell imaging platform for biomedical research and education for under $1750 [6]. This low-cost incubator also monitored and regulated temperature, CO_2, and humidity as per the parameters for successful mammalian cell culture. Past BME 200/300 design projects have attempted to build incubators for this client, but none have been completely successful.

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Appendix B: SOLIDWORKS CAD Drawing of the Proposed Cell Culture Incubator

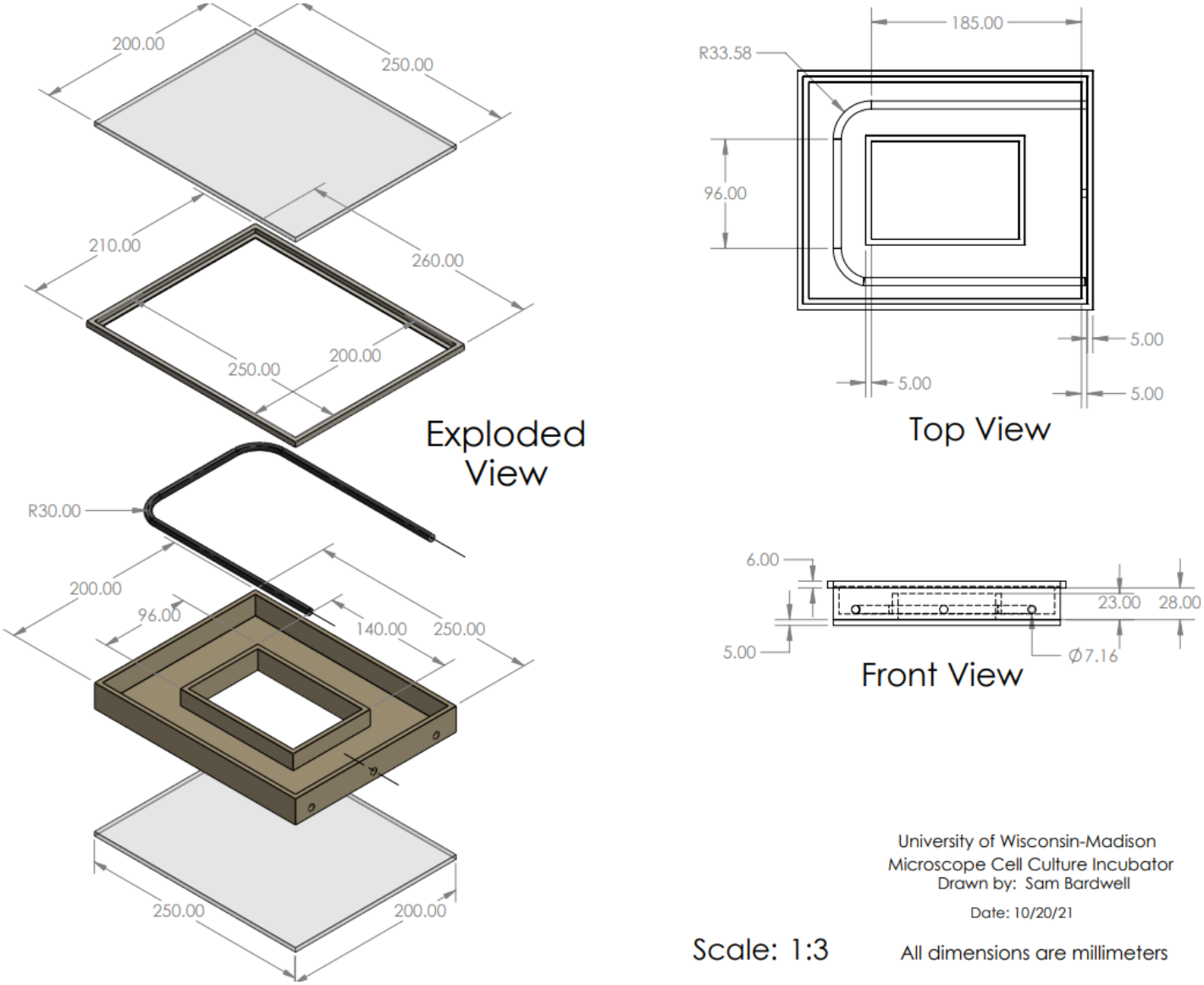
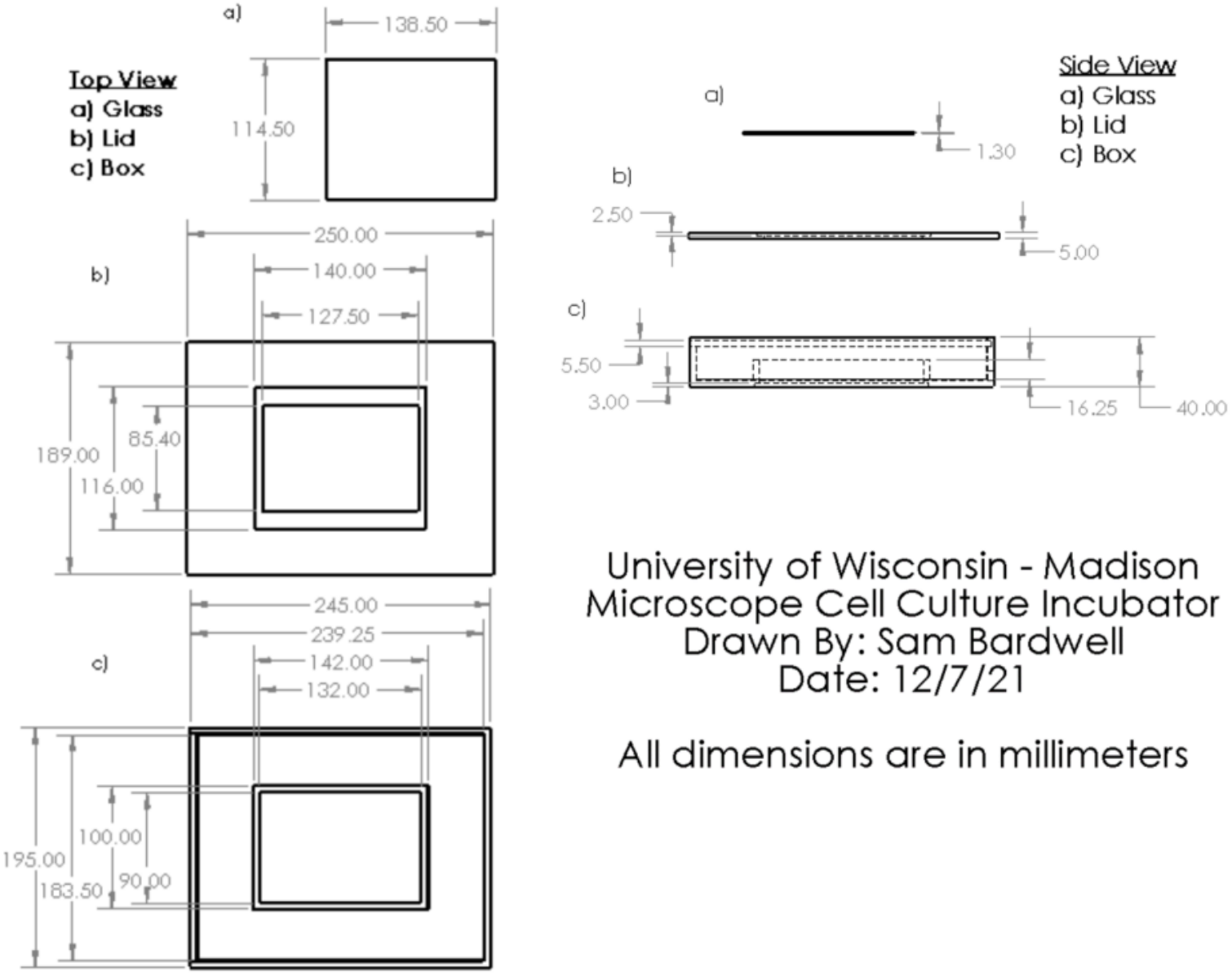
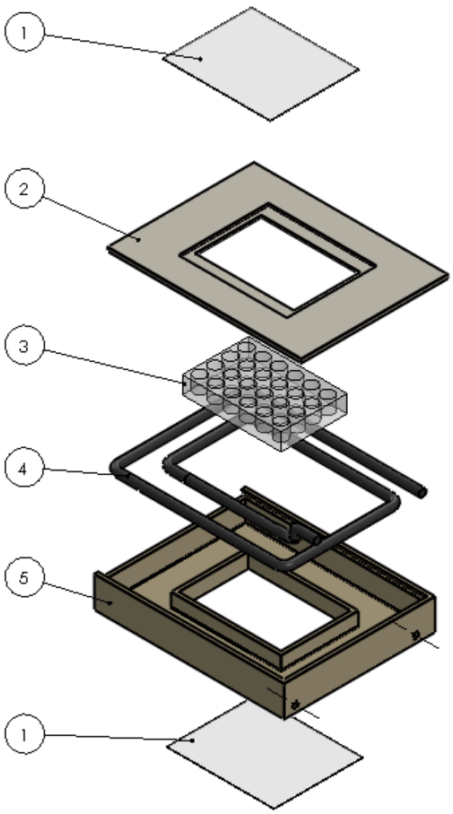


***Figure 1**: SOLIDWORKS Drawing of Design #2*

Appendix C: Final Design SOLIDWORKS Drawings and User Manual





Item No.	Item Description	Dimensions [mm]	QTY.
1	Glass plates to allow transparent viewing	114.5 x 138.5 x 1.3	2
2	Top removeable crown to allow access to interior	Outside: 189 x 250 x 5 Inside Cut: 85.4 x 127.5 x 2.5	1
3	Well Plate to hold cells	85.4 x 127.5 x 22.5	1
4	Inner heated water pump tubing	Outer Diameter: 12.7 Inner Diameter: 9.525	1
5	Incubator box to maintain a controlled internal environment	Outside: 195 x 245 x 40 Inside Cut: 90 x 132 19	1

Figure 1: Exploded SOLIDWORKS assembly of the final design along with a table explaining the dimensions and parts

Boot up Process

1) Remove sliding crown from incubator
2) Connect heated water pump tubing to the ribbed cone adaptor on incubator
3) Connect CO_2 tank hosing to incubator
4) Place incubator onto microscope shelf
5) Turn on heated water pump and set water temperature to 37° C
6) Fill incubator with enough DI water to submerge inner tubing
7) Turn on CO_2 tank and gauge to fill the internal environment to 5% CO_2 levels
8) Replace sliding crown back on the incubator
9) Allow time for internal environment to be set to 5% CO_2, 37° C, and 95-100% humidity
10) Compare desired inputs to the live sensor readings from the sensors

Inserting Well Plate

1) Slide open crown seal to expose well plate cavity
2) Insert a 138mm x 95mm or smaller well plate into designated cavity
    a) DO NOT use a well plate larger than dimensions given

3) Slide crown seal back into place on incubator
    a) Make sure seal is firmly in place
    b) DO NOT open until data acquisition is complete and sample isn't required anymore (will compromise internal environment otherwise)

Data Acquisition

1) Connect Arduino Microcontroller to a power source
2) Set up sensors to collect internal environment data
3) Upload designated code on Arduino IDE to print live internal environmental data
4) Record any desired values given by data

Cleaning and Disassembly

1) Make sure all power sources are disconnected
2) Empty DI water from inside
3) Remove external and inner tubing from incubator
4) Use ethanol to disinfect the inside of the incubator
    a) DO NOT use an autoclave because of the low melting points of the materials being used

Appendix D: Thermistor Circuit Diagram and Code

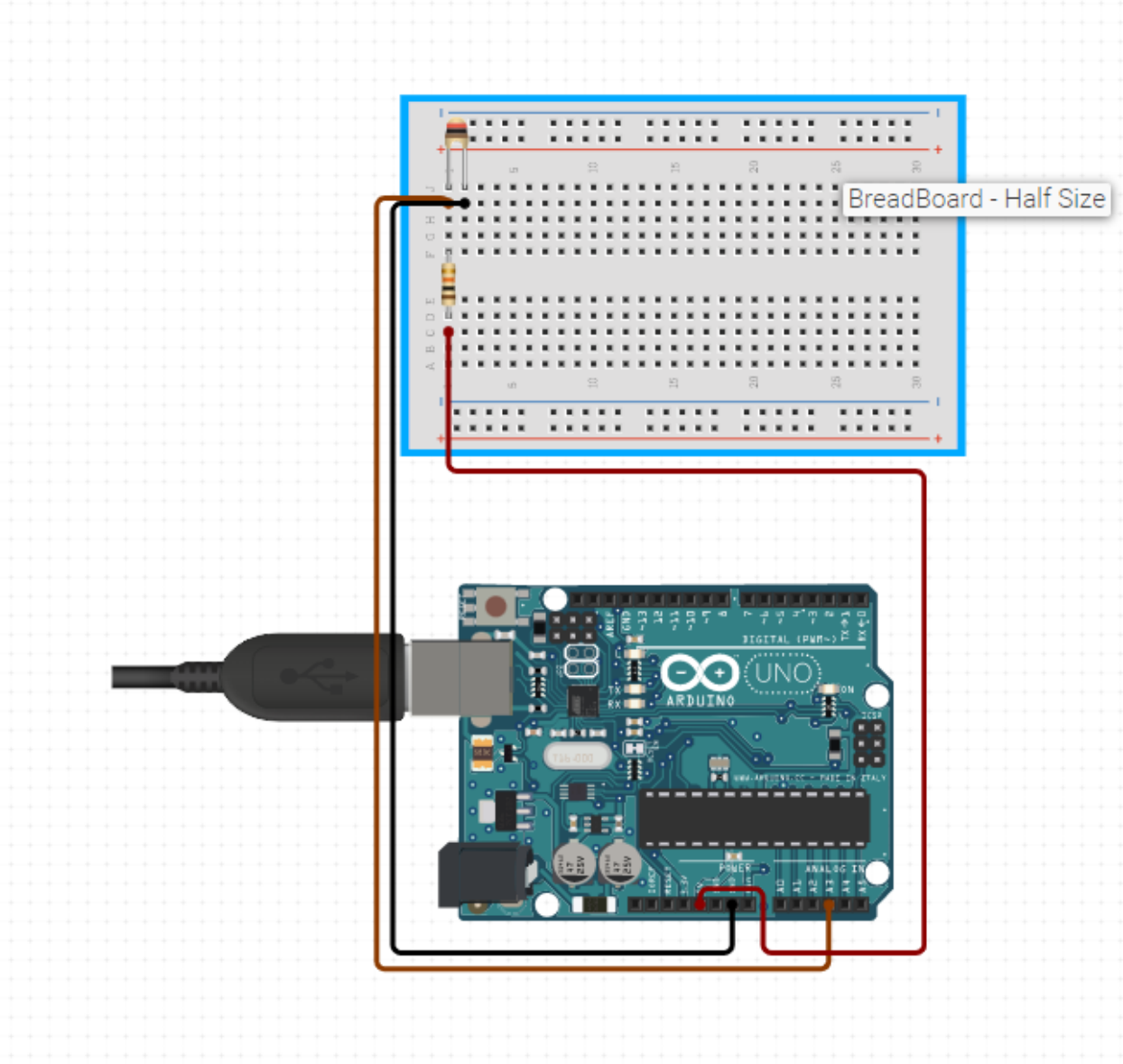


Figure 1: Thermistor Circuit Diagram

Arduino Code

```
int ThermistorPin = 0;
int Vo;
float R1 = 10000;
float logR2, R2, T, Tc, Tf;
float c1 = 1.009249522e-03, c2 = 2.378405444e-04, c3 = 2.019202697e-07;

void setup() {
Serial.begin(9600);
```

```
}

void loop() {

  Vo = analogRead(ThermistorPin);
  R2 = R1 * (1023.0 / (float)Vo - 1.0);
  logR2 = log(R2);
  T = (1.0 / (c1 + c2*logR2 + c3*logR2*logR2*logR2));
  Tc = T - 271.15;
  Tf = (Tc * 9.0)/ 5.0 + 32.0;
  float hum =0;

  hum = 100*exp((17.625*5.2)/(243.04+5.2))/exp((17.625*Tc)/(243.04+Tc)); //rel humidity
  Serial.print("Temperature: ");
  Serial.print(Tf);
  Serial.print(" F; ");
  Serial.print(Tc);
  Serial.println(" C");



  delay(500);
}
```

Appendix E: CO_2 Sensor Code and Circuit Diagram

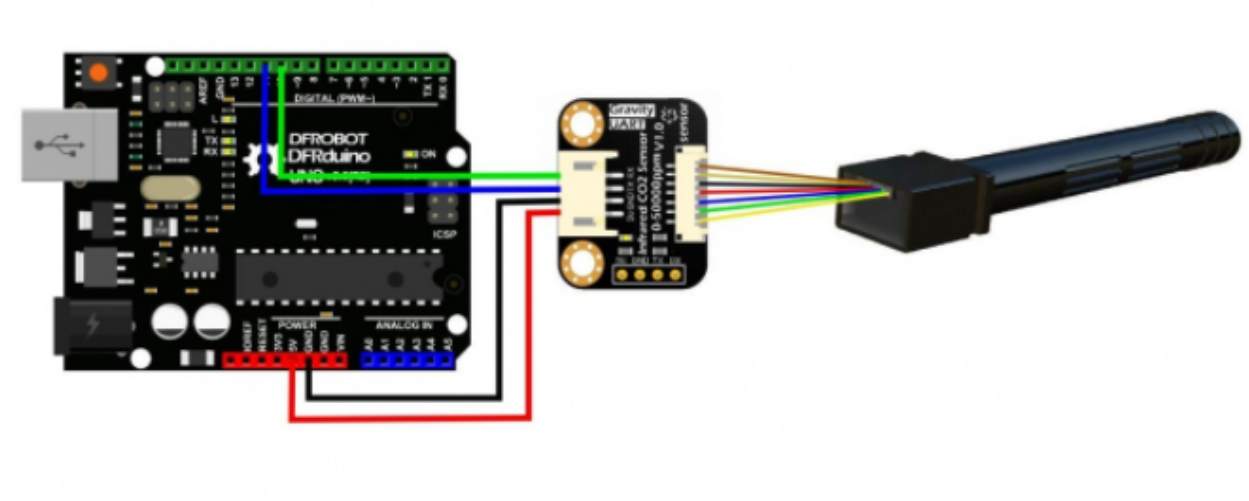


Figure 1: CO_2 Sensor Circuit Diagram [1]

Arduino Code

```
#include <SoftwareSerial.h>
#include <NDIR_SoftwareSerial.h>

//Select 2 digital pins as SoftwareSerial's Rx and Tx. For example, Rx=2 Tx=3
NDIR_SoftwareSerial mySensor(2, 3);
double percent = mySensor.ppm/10000;
void setup()
{
  Serial.begin(9600);

  if (mySensor.begin()) {
    Serial.println("Wait 10 seconds for sensor initialization...");
    delay(10000);
  } else {
    Serial.println("ERROR: Failed to connect to the sensor.");
    while(1);
  }

}

void loop() {
  if (mySensor.measure()) {
    Serial.print("CO2 Concentration is ");
    Serial.print(mySensor.ppm);
```

```
    Serial.println("ppm");
    Serial.print("Percent CO2 is ");
    Serial.print((mySensor.ppm/10000));
    Serial.println("%");
  } else {
    Serial.println("Sensor communication error.");
  }
 delay(1000);
  }
```

References

1. “Infrared CO2 Sensor ,” *DFRobot*. [Online]. Available: https://wiki.dfrobot.com/Infrared_CO2_Sensor_0-50000ppm_SKU__SEN0220. [Accessed: 01-Dec-2021].

Appendix F: Materials and Expenses

Table 1: Expenses

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Component 1: Incubator								
Transparent Cover Plates	top and bottom glass of incubator	Radnor	64005034	10/29	2	$1.04	$2.08	link
3D Printed Case	Sides and removable top of incubator	UW Makerspace	N/A	11/10	1	$32.32	$32.32	N/A
Component 2: Components								
1.5mm Tube Connector	connection between CO2 tank and incubator	Fisher Scientific	35031	10/29	1	$14.96	$14.96	LINK
3/8 and 1/4 in. Vinyl Tubing	heated water will flow through	USA Sealing	55YU99	11/23	1	$1.96	$1.96	LINK
Barbed Vacuum Connector	connection between tubing	Grainger	5ZMHI	11/23	2	$0.95	$1.90	LINK
½ by ⅜ in Vinyl tubing	Heated water will flow from machine to incubator	USA Sealing		12/7	1	$0.83/foot	$8.33	N/A
Cable Ties 4”	Used to secure tubing to connector and heated water pump	Ace Hardware	4027488	12/7	1	0.0828	$1.49	N/A
TOTAL:	$62.04							

Appendix G: Testing Protocols

Internal Environment - Temperature and Humidity Sensor Test Protocol (Thermistor +DHT)

Introduction

Name of Tester: Caroline Craig, Maya Tanna, Katie Day, Sam Bardwell
Dates of Test Performance: 11/29/2021 and 12/06/2021
Site of Test Performance: 1002 ECB

Explanation:

The team will be employing a sensor inside the incubator in order to measure the internal temperature. The measurements of the humidity and temperature will be obtained by an AOSONG DHT22 Arduino compatible sensor and a Thermistor. The team will test to make sure that the code and the AOSONG are working correctly by calibrating the sensor and then confirming its accuracy at steady state and precision in a dynamic range using a thermometer. To calibrate the sensor, the team will use resistance values on the Arduino Website. Once the sensor is calibrated, its accuracy will be tested by first measuring the temperature and humidity of the working environment to gauge if they are both working as expected, and then measuring its temperature at extreme high and low temperatures using a high dryer and freezer. Afterwards, the team will measure the temperature inside the incubator with a thermometer and the sensor. To keep the incubator completely sealed, the thermometer probe and reading display will be inserted into the incubator and read through the glass. The tests will be considered successful if the sensor value is within 2℃ of the thermometer temperature.

Steps	**Protocol**	**Verification/Validation**	**Pass/Fail**	**Initials of Tester**
1	Calibrate the sensor using resistance values on Arduino Website.	☑ ~~Verified~~ Comments:	Pass	CC, MT
2	Test the precision of the Arduino microcontroller at extreme high and low temperatures. Place the microcontroller in front of a hair dryer for two minutes and ensure the temperature outputs increase the longer it is under heat. Then, place the sensor in the freezer and ensure the temperature outputs decrease the longer it is under there. If the sensor follows these trends it is verified.	☑ ~~Verified~~ Comments:	Pass	CC, MT

3	Place the sensor in the lab incubator.	☑ ~~Verified~~ Comments:	Pass	KD, SB
4	Set up the Arduino sensor and incorporate the breadboard circuits.	☑ ~~Verified~~ Comments:	Pass	KD, SB
5	Record the average temperature of the system from the thermometer in the comments, taking measurements every 10 seconds over a period of 10 minutes. Verify that this temperature falls within the optimal range of 37 ℃ ± 2 ℃. **If the thermometer does not seem calibrated correctly, try first measuring the temperature of room temperature water (approximately 25 ℃).	☑ ~~Verified~~ Comments: Incubator was not able to get up to an optimal range of 37 ℃ ± 2 ℃.	Fail	KD, SB
6	Record the average temperature of the system from the Arduino microcontroller in the comments, taking measurements every 10 seconds over a period of 10 minutes. Verify that this temperature falls within ± 2 ℃ of the temperature read by the thermometer.	☑ ~~Verified~~ Comments: Thermistor was able to read values within ± 2 ℃ of the temperature read by the thermometer, however it was not at 37℃	Pass	KD, SB
7	Record the average humidity percentage from the Arduino microcontroller in the comments, taking measurements every 10 seconds over a period of 10 minutes, and verify that this value falls between 95-100%.	☑ ~~Verified~~ Comments: Thermistor code must be revised and also the temperature was not at optimal range.	Fail	KD, SB
8	Repeat steps 3-7, but place the sensor into the incubator prototype. Place a digital thermometer within the incubator to ensure the incubator stays sealed.	☑ ~~Verified~~	Fail	KD, SB

Internal Environment - CO_2 Sensor and Feedback System Test Protocol

Introduction

Name of Tester: Katie Day, Olivia Jaekle, and Sam Bardwell
Dates of Test Performance: 11/29/2021 and 12/06/2021
Site of Test Performance: 1002 ECB

Explanation:

The team will be employing sensors inside the incubator in order to measure the internal CO_2. For CO_2, the tank employed in the current lab has a sensor to check the CO_2 levels, but a CO_2 sensor will be placed inside the incubator as well. The measurement of CO_2 recorded by the Arduino sensors should be within 2% of the pressure gauge on the CO_2 tank.

Steps	**Protocol**	**Verification/Validation**	**Pass/Fail**	**Initials of Tester**
1	Test the precision of the sensor by ensuring its values increase and decrease with general increase and decrease of CO_2 concentration. Place the sensor in front of a CO_2 source. Ensure the sensor value readings increase as the sensor exposure to CO_2 gas increases. If this occurs, this step is verified.	☑ ~~Verified~~ Comments:	Pass	KD, OJ
2	Similarly, once the CO_2 supply is cut off, ensure the value readings from the sensor decrease. If this occurs, this step is verified.	☑ ~~Verified~~ Comments:	Pass	KD, OJ
3	Place the sensor into the lab incubator.	☑ ~~Verified~~ Comments:	Pass	KD, OJ
4	Record the CO_2 parts per million over a ten minute interval in the incubator. Ensure the values reach the values read by the incubator and fall within the range of ±0.1% CO_2.	☑ ~~Verified~~ Comments:	Pass	KD, OJ

5[6]	Set up the final incubator prototype for normal use. Record the value read by the fyrite at room conditions in the comments.	☐ Verified Comments:		
6	Set up the CO_2 sensor and fyrite within the incubator and seal it. Allow enough CO_2 to enter the incubator that the fyrite reads around 5% CO_2. Record the value given by the fyrite, the value given by the CO_2 sensor, and the trial number in the comments.	☐ Verified Comments:		
7	Remove the incubator from under the microscope and allow the CO_2 to leave the system so that its value read by the fyrite is nearly the same as room conditions. Repeat steps 5-4 until 5 trials are complete. Record the mean value of difference between the read CO_2 values in the comments.	☐ Verified Comments:		
8	If the CO_2 sensor deviates from the actual CO_2 percentage by ±0.1% or less, then the sensor is verified for use. If not verified, record why in the comments.	☐ Verified Comments:		

[6] CO_2 tank was not available for the completion of testing protocol

Steps	Protocol	Verification/Validation	Pass/Fail	Initials of Tester
1	Once the CO_2 sensor is approved for use, set up the incubator for normal use with the CO_2 sensor inside. Seal the incubator.	☐ Verified Comments:		
2	Connect the CO_2 tank to the incubator fixed with a regulator and a solenoid.	☐ Verified Comments:		
3	Verify the sensor is recording values. Then, begin running feedback code in conjunction with the solenoid connected to the CO_2 tank.	☐ Verified Comments:		
4	The solenoid should let CO_2 into the system immediately. Once the CO_2 sensor reads a value within 5% ±0.1% CO_2 the solenoid should stop allowing CO_2 into the incubator. If this occurs, continue protocol and step is verified. If this does not occur, stop protocol and record what happened in the comments.	☐ Verified Comments:		
5	Allow the feedback loop to run for an hour. Record the sensor values read into a graph. Verify that over the hour the CO_2 percentage remained near a level of 5% CO_2 ±0.1%. If the CO_2 remained in this range, continue protocol and step is verified. If this did not occur, stop protocol and record what happened in the comments.	☐ Verified Comments:		
6	Repeat step 5 over the course of 6 hours. If the CO_2 remains in the necessary range, continue protocol and step is verified. If this did not occur, stop protocol and record what happened in the comments.	☐ Verified Comments:		

Optical Testing - Prior to and After Installation

Introduction

Name of Tester: Caroline Craig and Maya Tanna
Dates of Test Performance: 11/29/2021
Site of Test Performance: 1002 ECB

Explanation:

The team will test High Transparent Lexan Polycarbonate sheets to determine which best matches the optical properties of well plates. Well Plates have a gloss percentage of 75-90, a haze percentage of 11, and a transparency percentage of 85-90 [16]. The team has researched that the transparency percentage of polycarbonate is 88-89 and the haze is 1%[17]. The team will determine through live cell imaging, either by fluorescent microscopy or bright field microscopy depending on the client's cell cultures, whether 88% transparency is acceptable.

Steps	Protocol	Verification/Validation	Pass/Fail	Initials of Tester
1	Have one team member complete steps 1-2. Prepare the microscope for use. Place prepared slide under a sheet of High Transparent Lexan Polycarbonate, and place onto the microscope stage.	☑ ~~Verified~~ Comments:	Pass	CC
2	Adjust the optical components of the microscope to best clarity based on personal judgement. Ensure the prepared slide is centered under the microscope lens. Take an image of what is observed under the microscope.	☑ ~~Verified~~ Comments:	Pass	CC
3	Repeat steps 1-2 without the polycarbonate sheet, but still including the prepared slide. Ensure the images are labeled with names that don't give away which image they are, but create a key for the names.	☑ ~~Verified~~ Comments:	Pass	CC
4	Have 3 team members, other than the one who completed steps 1-3, complete this step. Have each	☑ ~~Verified~~ Comments:	Pass	MT

	member choose which image they believe is clearer, or if they look the same. Record the members' responses in the comments.			
5	If the majority can see a difference in clarity between the two images, the test has failed and a different clear material should be tested for use.	☑ ~~Verified~~ Comments:	Pass	MT

Steps	**Protocol**	**Verification/Validation**	**Pass/Fail**	**Initials of Tester**
1	Prepare the microscope for use. Get internal conditions of the incubator to those needed for live-cells.	☑ ~~Verified~~ Comments:	Pass	MT
2	Place mammalian cells provided by the client in the incubator. Place the incubator onto the microscope stage.	☑ ~~Verified~~ Comments:	Pass	MT
3	Adjust the optical components of the microscope to best clarity based on personal judgement. Take an image of what is observed under the microscope.	☑ ~~Verified~~ Comments:	Pass	MT
4	Repeat steps 1-3 without the polycarbonate sheets, but still including the cells.	☑ ~~Verified~~ Comments:	Pass	MT
5	Have client assess the visual clarity of the images in a blind fashion. Record their ranking from 1-10 based on 10 being the best. Record results in comments with a 9-10 being a pass. Additionally, have the client assess the distortion of the images and record their response.	☑ ~~Verified~~ Comments:	Pass	MT
6	Using ImageJ, record the clarity of the images using the microscope focus quality plugin. The images	☑ ~~Verified~~ Comments:	Pass	MT

	will be divided into regions and assigned a color based on their focus level. Compare these images and their similarity.			

Recovery Test Protocol

Introduction

Name of Tester:
Dates of Test Performance:
Site of Test Performance:

Explanation:

The team will test the recovery time of the incubator after it has been opened by timing how long it takes for the incubator to return to performance conditions (37°C, 5% CO_2, and >95% humidity). The maximum recovery time should not exceed five minutes after a 30 second exposure to the external environment.

Steps	**Protocol**	**Verification/Validation**	**Pass/Fail**	**Tester Initials**
1	Set up the incubator for normal use. Record internal conditions in the comments and verify that they fall within the correct ranges (37°C, 5% CO_2, and >95% humidity).	☐ Verified Comments:		
2	Open the incubator for 30 seconds. Start stopwatch. Verify that the stopwatch is working.	☐ Verified Comments:		
3	Record internal conditions in the comments at a time of 15 seconds after opening the incubator. Verify that the internal conditions deviate from the normal conditions recorded above.	☐ Verified Comments:		
4	Close the incubator. Verify that the recovery time did not exceed 5 minutes after a 30 second exposure to the external environment. Record the time it took to revert back to optimal conditions in the comments.	☐ Verified Comments:		