Microscopic Cell Culture Incubator
Preliminary Report

BME 301 Design
March 2nd 2022

Client: Dr. John Puccinelli
University of Wisconsin-Madison
Department of Biomedical Engineering

Advisor: Dr. Melissa Kinney
University of Wisconsin-Madison
Department of Biomedical Engineering

Team:
Leader: Sam Bardwell
Communicator: Katie Day
BWIG: Maya Tanna
BSAC: Bella Raykowski
BPAG: Drew Hardwick

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 incubator. There are current designs on the market that meet this criteria, but either the inverted microscope is encapsulated into the incubator making it bulky and inconvenient to disassemble, or the incubator is very expensive. The team is going to design a cost-effective 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.

Table of Contents

Body of Report

I. Introduction

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 duration of the study. Onstage 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 caused 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 guidelines[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].

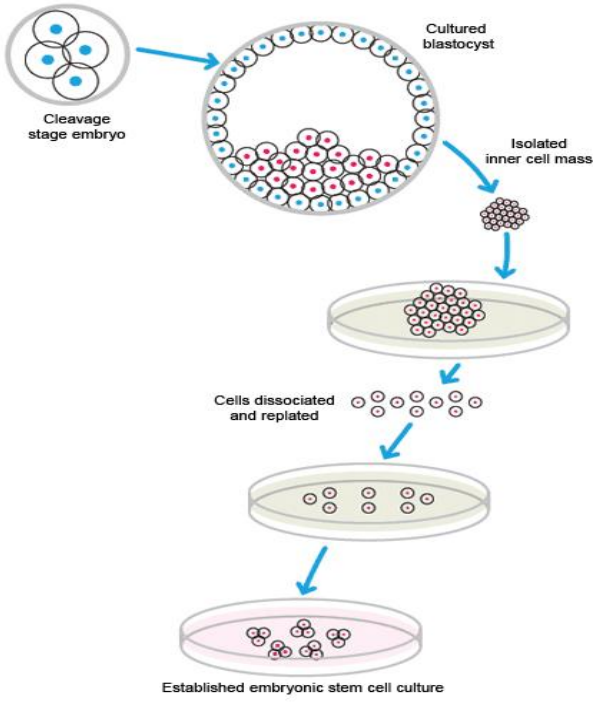


Figure 1: Isolation of Embryonic Stem Cell Lines[7]

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' environmental conditions in the body (37℃ with a pH of 7.2-7.4) [8]. 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]. Many incubators are therefore larger in size in order to maintain these homeostatic conditions. However, there are some commercially available stage top incubators that are able to adhere to the specifications required to keep cells viable, but they are often more expensive. See Appendix A for more information regarding these competing designs.

Figure 2: Thermo Fisher Incubator [9]

Incubator Types

There are two types of commonly used methods to maintain temperature in industry cell incubators. Many employ the direct heat method which tends to give off heat using electric metal coils that surround the body of the incubator, and are 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 taken over a period of time. Pharmaceutical companies can also access the drug cytotoxicity in different cell types. Virology and vaccine products benefit from live cell cultures as they 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 genetic engineering/gene therapy

applications of these cell cultures, and the expression of specific genes and the impact they have on other cells can be studied.

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 their teaching lab where students will conduct live cell imaging on tissues for up to one week at a time. The specifics of the experiment are unknown, however it is believed that this device will 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 to the scope or disassembling a bulky microscope assembly allowing more time to 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% ± 0.5% 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 fit on an inverted microscope stand (roughly 310 x 300 x 45mm) without interfering with the microscope's optics and functionality. The device must also be able to hold a standard well plate (127.55 x 85.4 x 22.5mm) without disrupting the integrity of the plate of cultures in the plate. 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 low-cost, easily assembled/disassembled, sterilized, and can be easily moved and stored 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.

Successes of Fall 2021

This project was worked on previously by many BME 200/300/400 students, however last semester, Fall 2021, brought the most success to the project. The team, consisting of continuing members Maya Tanna, Sam Bardwell, and Katie Day and others, was able to create an incubation chamber out of PLA plastic with working temperature and humidity sensors. The incubation chamber was 195 x 245 x 40 mm with a vinyl tubing, inner diameter of ¼ inch and outer diameter of ⅜ inch, was wrapped around the interior of the box. The vinyl tubing was connected to nylon barbed vacuum connectors , ⅜ x ⅜ inch, which was then hooked up to a heated water pump. The interior also contained a small water bed, roughly 1 liter in volume, that in theory should be heated via thermal conductivity of the vinyl tubing induced by the flowing heated water from the pump. However, the results of last semester proved that vinyl tubing did

not have the right thermal conductivity to heat the water bed to higher than 20℃. The temperature and CO_2 sensors were coded and tested, both of which proved that the code ran smoothly and was able to accurately measure the internal environment of the incubator. Last semester, incorporation of CO_2 into the chamber was not possible, however it is of the utmost importance this semester.Appendix B contains more relevant information on the previous semesters work.

Figure 3: Incubation Chamber Fall 2021

III. Preliminary Designs

Design #1 Hinge Top Acrylic Incubator

The hinge top acrylic incubator (Figure 4) consists of a 245x195x40mm black acrylic box with three feet of copper tubing circling the inner box twice in order to provide heat via conduction. The hinge top incubator received its name because the lid of the incubator will be placed on a rubber lining on top of the main box. When the lid is placed on top, hinges around the box will hook onto the lid and then be clamped down to compress the lid providing a tight seal for the internal environment. The mechanism will be similar to a hinge and latch on a tackle box used in fishing. The black acrylic will be designed in SOLIDWORKS with the ability to be laser cut to increase precision, decrease cost, and to expedite the fabrication process. A couple downfalls to this design is with the addition of the latches. There will be more risk of fracturing the acrylic tabs that the latches will hook onto and the latches will increase the amount of fabrication steps.

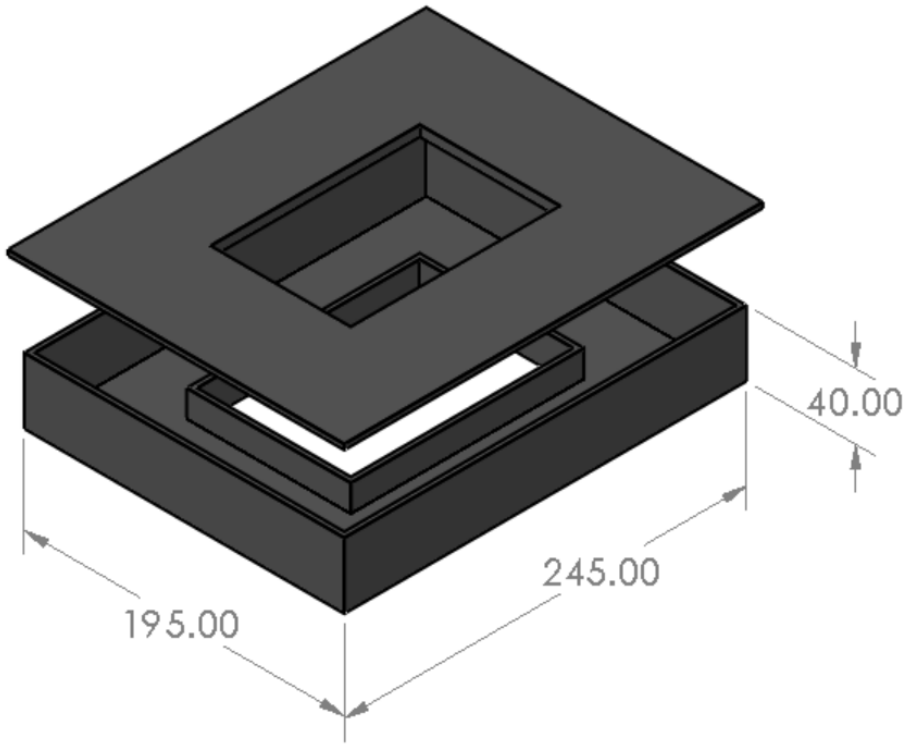


Figure 4: Solidworks Image of Preliminary Design #1 (all dimensions in mm)

Design #2 Slide Top Acrylic Incubator

The slide top acrylic incubator (Figure 5) will be made of laser cut, black acrylic, similar to Design #1. The physical design will be comparable to the previous semester work (Appendix B) with a change in material and fabrication process. The slide top acrylic incubator lid will be able to slide into and out of a slit carved into the inside of the main box. This will allow for easy access into the well plate of the incubator, without disrupting the entire internal environment. This design will include the three feet of copper tubing circling the inner box twice in order to provide the 37°C temperature values via conduction. One downfall to this design is the slide top provides more areas for there to be loss of the internal environment. If the slide top slit isn't a perfect fit, there will not be a perfect seal between the cover and box causing fogging of the glass impacting the optical clarity.

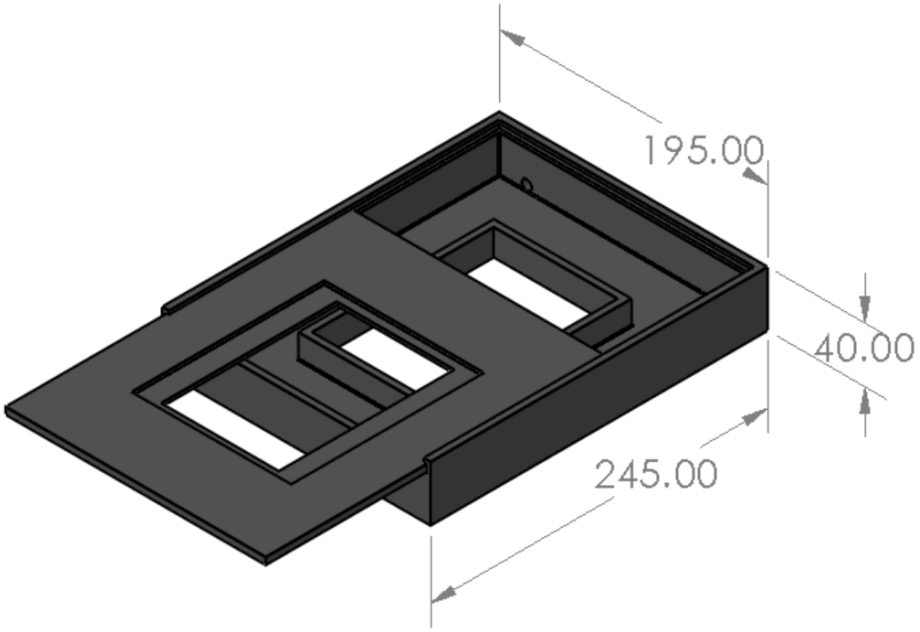


Figure 5: Solidworks Image of Preliminary Design #2 (all dimensions in mm)

Design #3 3D Printed Incubator

The 3D printed incubator (Figure 6) will be made with the same SOLIDWORKS drawings as the previous semester. The box will be made of white PLA plastic with an inner coating of flex seal, insulation spray, or liquid concrete. The box will have the slide top concept to allow easy access to the inside of the incubator. The inner box will be wrapped with the same three feet of copper tubing as the previous two designs to maximize the heat transfer between the heated water pump and the inner water bed. A couple downfalls to this design is the cost, material properties, and sealant capabilities. 3D printing is much more expensive and with the addition of an extra sealant to prevent the PLA plastic from leaking or cracking, the cost will add up quickly. The one benefit to 3D printing is the minimal fabrication.

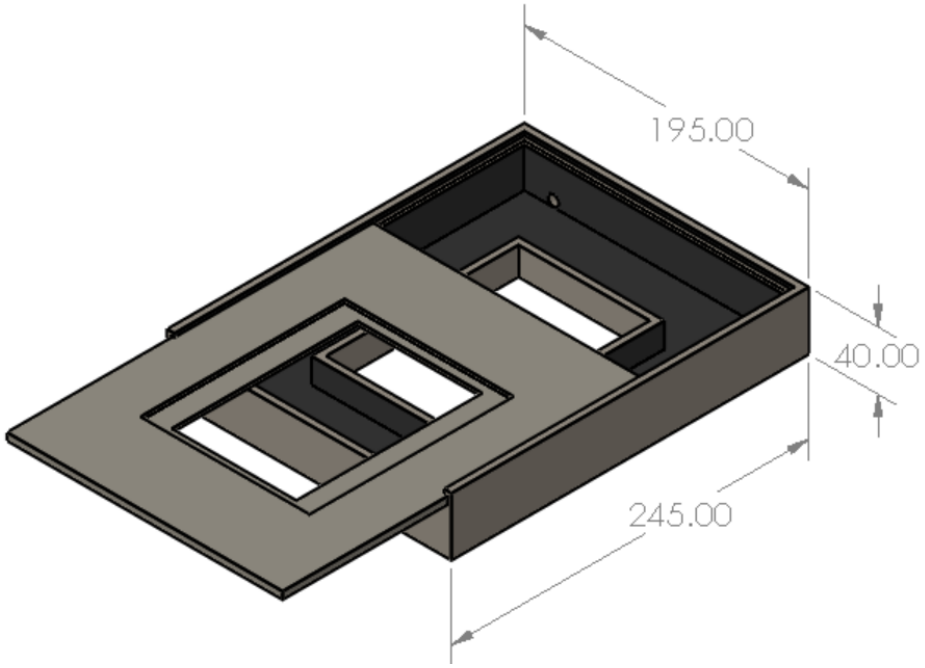
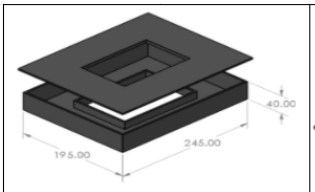
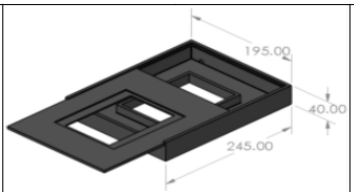
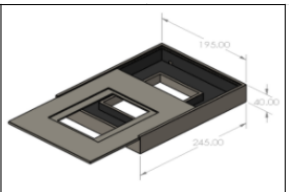


Figure 6: Solidworks Image of Preliminary Design #3 (all dimensions in mm)

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.

								
			Hinge Top Acryllic Incubator		**Slide Top Acryllic Incubator**		**3D Printed Incubator**	
Rank	Criteria	Weight	Score (5 max)	Weighted Score	Score (5 max)	Weighted Score	Score (5 max)	Weighted Score
1	Internal Environment	25	5	25	4	20	4	20
2	Microscope Compatibility	20	5	20	5	20	5	20
3	Accuracy and Reliability	20	4	16	4	16	3	12
4	Ergonomics	15	5	15	5	15	5	15
5	Cost	10	4	8	4	8	3	6
6	Life in Service	5	5	5	5	5	4	4
7	Safety	5	5	5	5	5	5	5
	Sum	**100**	**Sum**	**94**	**Sum**	**89**	**Sum**	**82**

* All box dimesions are in millimeters

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% ± 0.5% 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 is deciding to move forward with Design #1, the hinge top acrylic incubator. Since the material will be laser cut acrylic at the UW Makerspace, the cost will be lower than 3D printing. This incubator will also provide the best internal environment and will reduce the majority of leakage throughout the incubator because of the rubber lining addition. With the addition of the inner copper tubing, the heat transfer between the heated water pump water to the water bed will be maximized, resulting in the incubator being able to reach the 37℃ temperature as well as the desired humidity of >95%. The incubator will be paired with a 100% CO_2 input with sensor readings increasing or decreasing the amount of gas being inserted. Temperature, humidity, and CO_2 sensor coding and circuitry will be improved from the previous semester to provide more accurate and precise data readings. Overall, the first design allows for the most

compatibility and improvement of materials, accuracy, and design criteria compared to the other designs.

V. Fabrication/Development Process

Materials

Arduino Materials

The circuitry 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 will use an equation (see Appendix B) to determine the relative humidity inside the incubator. The accuracy of this equation will be tested against the DHT22 temperature and humidity sensor.

In order to measure CO_2 levels inside the incubator, the team will use 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. A valve that can be connected to a CO_2 tank will be utilized to control the CO_2 input into the incubator. The flow of CO_2 will be monitored via a DC motor with a motor arm attachment that will be controlled by the Arduino microcontroller. The DC motor will twist the valve left or right to let in more or less CO_2 depending on the NDIR sensor values.

Incubator Materials

The incubator will be equipped with approximately 3ft of copper tubing to allow for heat transfer. The copper tubing will allow for sufficient heat to be conducted to the 1L waterbed that will sit inside the proposed final design to allow for both optimal temperature and humidity. The incubator will be made using black acrylic from the UW-Makerspace. The acrylic was chosen as an alternative to the PLA plastic used last semester for the prototype. Black acrylic has a larger ultimate tensile strength (70MPa) than PLA, is cheaper, and the black allows for more insulation and protection from light [16]. Dr. Puccinelli also informed the team that a black acrylic box would be compatible with a fluorescent microscope, as well as an inverting microscope, should the incubator be used in other projects in the future.

Methods

Fabrication of the prototype will begin with an 18x24x ⅛ inch sheet of black acrylic plastic. The acrylic sheet will be placed onto the UW Madison Makerspace laser cutter. The box will be laser cut 2-dimensionally using a CAD drawing in SOLIDWORKS and converting the drawing to the laser cutter language. Once the individual pieces are cut out, the team will use acrylic glue to build the box 3-dimensionally.

Additional materials such as latches, copper tubing, glass, CO_2 input, and sensors will be incorporated into the box once it is built. The latches will be glued onto the side of the acrylic box in a way such that the clamps will force the lid to be compressed onto the rubber lining. The inner copper tubing will be connected to the heated water pump tubing with a metal adaptor. The glass will be glued onto its corresponding indents in the bottom of the box and the lid to allow for transparent viewing. A valve that can be connected and disconnected to an outsourced CO_2 tank will be built into the side of the incubator, and controlled from a DC motor. The motor will be connected to an Arduino microcontroller that contains code reading values from the NDIR CO_2 sensor, to limit/regulate the amount of CO_2 in the well. The DC motor is needed because the team has decided to use a 100% CO_2 tank in order to meet the budget requirements. Lastly, the sensors will be inserted into the same spots as the previous semester to collect live data on the values of temperature, humidity, and percent CO_2.

The thermal conductivity of copper was assessed along with the heat transfer rate of copper measure by using equation 1[17].

$$Q = mCp\Delta T \quad \text{[kJ]} \ (1)$$

Using this it was determined that if the heated water pump pushes water out at an initial temperature of 50℃, the 1L water bed will reach the desired temperature of 37℃, starting from 20℃, within 7.4 minutes. Once the desired temperature is reached, the heated water pump will be set to 38℃ in order to maintain a 37℃ internal temperature and to account for any loss of heat throughout the vinyl tubing of the water pump and acrylic walls of the incubator.

The Arduino sensing unit will be developed using the materials recommended by the Arduino website in order to build a basic circuit that has both temperature and CO_2 testing. The team will use the sample code provided by Arduino with some minor modifications in order to also output the humidity readings.

Final Prototype

Final prototype has not been fabricated yet.

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 C 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 will be evaluated using the Internal Environment - Temperature and Humidity Sensor Testing Protocol[1]. First, the sensor will be calibrated using resistance values given by the Arduino website. Once the sensor is calibrated, its precision in a dynamic range will be 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 or heated water cup and freezer.

Next, the accuracy of the thermistor will be evaluated by placing it into the lab incubator and ensuring it reads the temperature the incubator is set to within an error range of ± 0.5°C. After placing the sensor in the lab incubator for 10 minutes, the temperature reading will be ensured to accurately record the incubator temperature over the entire time interval.

Finally, the temperature sensor will be tested within the microscope cell culture incubator itself. The incubator will be set up for normal use, and the sensor and a digital thermometer will be placed within the incubator before it is sealed. The ability for the incubator to maintain a temperature of 37°C ± 0.5°C will be 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 will be 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 are passed, the thermistor and the incubator's ability to maintain the temperature internal conditions will be approved. If any of these tests are not verified, then the incubator will be reassessed at that point and testing will be redone before approval.

CO_2 Testing

The ability for the CO_2 sensor to accurately record whether the incubator maintains an internal environment of 5% ± 0.5% will be evaluated using the Internal Environment - CO_2 Sensor and Feedback System Testing Protocol. Once the sensor is calibrated, its precision in a dynamic range will be evaluated by ensuring its values increase and decrease with general increase and decrease of CO_2 concentration. The sensor will first be tested in room conditions to ensure it gives a consistent reading. Then, the sensor will be exposed to an increased concentration of CO_2 by having group members breathe on the sensor and the sensor readings will be observed to ensure it increases in value. Similarly, the CO_2 supply will be cut off and a

[1] See Appendix C for Testing Protocols

decrease in concentration readings from the sensor will be verified. If the sensor increases and decreases in CO_2 percentage readings as expected, then its precision in a dynamic range will be approved.

Next, the accuracy of the CO_2 sensor will be evaluated by placing it into the lab incubator and ensuring it reads the concentration the incubator is set to within an error range of ± 0.5%. After placing the sensor in the lab incubator for 10 minutes, the CO_2 sensor reading will be ensured to accurately record the incubator temperature over the entire time interval.

Finally, the CO_2 sensor will be tested within the microscope cell culture incubator itself. The incubator was set up for normal use, and the sensor and a fyrite will be placed within the incubator before it is sealed. The ability for the incubator to maintain a concentration of 5% CO_2 ± 0.5% will be 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 will be evaluated by taking measurements every 10 seconds over a period of 10 minutes and verifying the sensor records concentration values of 5% CO_2 ± 0.5%.

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

Optical Testing

The optical clarity of the Transparent Polycarbonate sheets will be evaluated qualitatively and quantitatively to ensure they do not impair the microscope's ability to view the cell culture. First, the sheets will be evaluated qualitatively. The microscope and its imaging software will be prepared for use. Then, one team member will place a prepared slide under a sheet of the High Transparent Lexan Polycarbonate and place those two onto the microscope stage. The microscope will then be adjusted to the best clarity and an image of what is observed under the microscope will be captured. The same procedure will then be followed but without the Polycarbonate sheet. To ensure the images quality could be evaluated in a blind and objective fashion, the tester will label the images and create a key for the naming process. Finally, three team members who are not present for the imaging process will assess the clarity of the two images. Each member will choose which image they believe is clearer, or if they look 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 will be evaluated quantitatively. The microscope and its imaging software will be prepared for use, and then the same imaging process from before will be 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 will be recorded; the images will be divided into gridded squares and each square will be assigned a color based on their focus level. The

assessments of each image will then be compared to evaluate their similarities in clarity. If the majority of the regions in both images are the same, then the Polycarbonate sheets passed the quantitative test and will be 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. This recovery testing will ensure that the incubator system can return to optimal homeostatic levels after there is a disruption in the system, validating the effectiveness of the device.

VI. Results (Future Work for Now)

Now that a final design has been proposed, the prototyping and testing stages of the project can begin. The group plans to break into three teams Materials/CO_2, Arduino Coding, and Incubator Fabrication which will each work independently to streamline the design process. The materials group will determine and purchase necessary materials along with determining the correct method to accurately measure and monitor CO_2 input. The incubator fabrication group will begin prototyping and creating testing protocols. The Arduino coding group will begin writing and testing their code for the sensors.

VII. Discussion

Discussion will be written once results have been collected.

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 a design that is lightweight, cost-effective, and able to maintain the desired internal environment. The proposed final design will include a copper tube that is wrapped around the inside of the

incubator and connected to a heated water pump that will regulate the internal incubator conditions and keep them at their optimal values. The lid to the incubator will be a hinge top which will allow for a tighter seal of the internal environment and help prevent leakage. The incubator box will also contain a hole for CO_2 to be pumped in, a CO_2 sensor, and thermistor temperature sensor that will in addition be coded to calculate the internal humidity. The CO_2 input will be monitored using a DC motor that receives direction from the NDIR sensor via Arduino coding. Moving forward, the team will begin the prototyping and purchasing stages of the design process, before moving onto the testing phase.

IX. References

1. C.-P. Segeritz and L. Vallier, “Cell Culture,” Basic Science Methods for Clinical Researchers, pp. 151–172, 2017, doi: 10.1016/B978-0-12-803077-6.00009-6.
2. “CO2 Incubators Market | Growth of Global Life Science Market to Boost the Market Growth | Technavio,” Oct. 10, 2020. https://www.businesswire.com/news/home/20201009005417/en/CO2-Incubators-Market-Growth-of-Global-Life-Science-Market-to-Boost-the-Market-Growth-Technavio (accessed Oct. 19, 2021).
3. “Enviro-Genie - Scientific Industries, Inc.” https://www.scientificindustries.com/enviro-genie.html?gclid=CjwKCAjwkvWKBhB4EiwA-GHjFoukLkKG-Gvoq4OtC7PgR6UgSMcVMjsQiUTasRU_aDfpk6TYdgopABoCM1wQAvD_BwE (accessed Oct. 19, 2021).
4. “Cell Culture - ScienceDirect.” https://www.sciencedirect.com/science/article/pii/B9780123741448000485 (accessed Oct. 19, 2021).
5. “Biosafety Levels 1, 2, 3 & 4 | What’s The Difference?,” Consolidated Sterilizer Systems, Apr. 14, 2015. https://consteril.com/biosafety-levels-difference/ (accessed Oct. 19, 2021).
6. P. Hannifin and D. Hunter, “Introduction to ISO Air Quality Standards.” pp. 1–12, 2010.
7. https://www.bio-rad.com/en-us/applications-technologies/isolation-maintenance-stem-cells?ID=LUSR1TC4S
8. I. K. Hartmann and J. Wagener, “CO2 Incubators – Best Practices for Selection, Set-up and Care,” p. 10.
9. “CO2 incubators,” Thermo Fisher Scientific - US. [Online]. Available: https://www.thermofisher.com/us/en/home/life-science/lab-equipment/co2-incubators.html. [Accessed: 20-Sep-2021].
10. “Introduction to Cell Culture - US.” //www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/introduction-to-cell-culture.html (accessed Oct. 19, 2021).
11. N. Pauly, B. Meuler, T. Madigan, and K. Koesser, “Microscope Cell Culture Incubator ,” BME Design Projects , 22-Apr-2021. [Online]. Available: https://bmedesign.engr.wisc.edu/projects/s21/scope_incubator/file/view/8badf1ad-7028-4c7c-9cb5-79cc22fe65da/BME%20Final%20Poster.pdf. [Accessed: 03-Oct-2021].
12. N. Pauly, T. Madigan, K. Koesser, and B. Meuler, “Microscope Cell Culture Incubator - bmedesign.engr.wisc.edu.” [Online]. Available: https://bmedesign.engr.wisc.edu/projects/f20/scope_incubator/file/view/db2b6829-fcc8-4732-8cec-94e60a3cc722/Final%20Report.pdf. [Accessed: 03-Oct-2021].

13. N. Pauly, B. Meuler, T. Madigan, and K. Koesser, “Microscope Cell Culture Incubator ,” BME Design Projects , 22-Apr-2021. [Online]. Available: https://bmedesign.engr.wisc.edu/projects/s21/scope_incubator/file/view/8badf1ad-7028-4c7c-9cb5-79cc22fe65da/BME%20Final%20Poster.pdf. [Accessed: 03-Oct-2021].
14. “CFR - Code of Federal Regulations Title 21,” accessdata.fda.gov, 01-Apr-2020. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=864.2240. [Accessed: 20-Sep-2021].
15. “Department of Labor Logo United Statesdepartment of Labor,” Law and Regulations | Occupational Safety and Health Administration. [Online]. Available: https://www.osha.gov/laws-regs. [Accessed: 07-Oct-2021].
16. “Young's modulus, tensile strength and yield strength values for some materials,” Engineering ToolBox, 2003. [Online]. Available: https://www.engineeringtoolbox.com/young-modulus-d_417.html. [Accessed: 25-Feb-2022].
17. Thermal Physics, “Measuring the Quantity of Heat,” The Physics Classroom, 2021. [Online]. Available: https://www.physicsclassroom.com/class/thermalP/Lesson-2/Measuring-the-Quantity-of-Heat#:~:text=Q%20%3D%20m%E2%80%A2C%E2%80%A2,temperature%20change%20of%20the%20object. [Accessed: 25-Feb-2022].
18. “ISO 13485:2016,” ISO, 21-Jan-2020. [Online]. Available: https://www.iso.org/standard/59752.html. [Accessed: 20-Sep-2021].
19. A. Trapotsis, “Biosafety levels 1, 2, 3 & 4: What's the difference?,” Consolidated Sterilizer Systems, 01-Apr-2020. [Online]. Available: https://consteril.com/biosafety-levels-difference/. [Accessed: 20-Sep-2021]

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 (less than 310 x 300 x 45mm[1]), 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. This device should demonstrate no quantitative difference on the microscope when adding glass compared with solely cells, in order to demonstrate full transparency of the top and bottom slides of the system.

b. ***Safety:*** The incubator and the cell culture environment must be in cooperation with BioSafety Level 1 Standards [2]. 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 ± 0.5°C throughout the entire internal environment. The humidity must be kept above 95% humidity. CO_2 levels must be 5% ± 0.5%. The incubator must be able to maintain these conditions constantly for at least two weeks. The device must also be able to reach these conditions after the incubator has been opened and exposed to the external environment within five minutes of interruption.

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 for at least two weeks, without compromising the integrity of the microscope's optics or functionality. Measures must be taken

to ensure that the temperature is the same in all areas of the chamber with an error of ± 0.5°C The box also must be sealed efficiently 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 device must be less than 310x300x45mm in order to fit on the microscope stand without interfering with the optics[1].The bottom and top of the incubator will be 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 transportability 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, are water tight, 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 financed 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 [3]. 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 [4].

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. Both okolabs and Elliot Scientific have stage-top microscopic incubators available, both of which use the direct heat method, and have had great success in maintaining a homogeneous environment in terms of temperature and CO2 percentage[6,7]. However, these stage top incubators are still extremely expensive ranging from $431-$1000 and are only compatible with XY stage inserts[8]. XY stage inserts are roughly 150x150x36mm[9], slightly smaller dimensions than the stage top the team is currently working on. 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 [10]. This low-cost incubator also monitored and regulated temperature, CO2, 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.

References

1. “Nikon Eclipse Ti-S Inverted Phase Contrast,” *Cambridge Scientific*, 2022. [Online]. Available: https://www.cambridgescientific.com/used-lab-equipment/product/Nikon-Eclipse-Ti-S-Inverted-Phase-Contrast-Fluorescent-Microscope-16358. [Accessed: 09-Feb-2022].
2. A. Trapotsis, “Biosafety levels 1, 2, 3 & 4: What's the difference?,” Consolidated Sterilizer Systems, 01-Apr-2020. [Online]. Available: https://consteril.com/biosafety-levels-difference/. [Accessed: 20-Sep-2021].
3. “ISO 13485:2016,” ISO, 21-Jan-2020. [Online]. Available: https://www.iso.org/standard/59752.html. [Accessed: 20-Sep-2021].
4. “CFR - Code of Federal Regulations Title 21,” accessdata.fda.gov, 01-Apr-2020. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=864.2240. [Accessed: 20-Sep-2021].
5. “Average Cost of Cell Culture Incubator ,” Google shopping. [Online]. Available: https://www.google.com/search?q=average%2Bcost%2Bof%2Ba%2Bcell%2Bculture%2Bincubator&sa=X&rlz=1C1CHBF_enUS919US919&biw=1309&bih=882&tbm=shop&tbs=mr%3A1%2Cp_ord%3Apd%2Cnew%3A1&ei=OQBJYe-2GuiO9PwPpcK6sAg&ved=0ahUKEwivt7G9wo7zAhVoB50JHSWhDoYQuw0IjwUoAw. [Accessed: 20-Sep-2021].
6. “CO2 incubators,” Thermo Fisher Scientific - US. [Online]. Available: https://www.thermofisher.com/us/en/home/life-science/lab-equipment/co2-incubators.html. [Accessed: 20-Sep-2021].
7. Okolab, “Stage Top Chamber,” *Okolab - stage top - digital gas*, 2003. [Online]. Available: http://www.oko-lab.com/live-cell-imaging/stage-top-digital-gas. [Accessed: 23-Feb-2022].
8. “Microscope Incubation Systems,” *Elliot Scientific Website*, 2020. [Online]. Available: https://www.elliotscientific.com/DPMH-Microscope-Incubators. [Accessed: 23-Feb-2022].
9. “XY mechanical measurement stage for microscopes + digital micrometer head,” *BoliOptics*, 2022. [Online]. Available: https://bolioptics.com/xy-mechanical-measurement-stage-for-microscopes-digital-micrometer-head/#:~:text=XY%2DAxis%20Drive%20Mode%3A%20Manual,Stage%20Height%3A%2036mm. [Accessed: 23-Feb-2022].
10. M. P. Walzik, V. Vollmar, T. Lachnit, H. Dietz, S. Haug, H. Bachmann, M. Fath, D. Aschenbrenner, S. A. Mofrad, O. Friedrich, and D. F. Gilbert, “A portable low-cost long-term live-cell imaging platform for Biomedical Research and Education,” Biosensors and Bioelectronics, 28-Sep-2014. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0956566314007489. [Accessed: 20-Sep-2021].

Appendix B: Incubator Fall 2021

Final Design

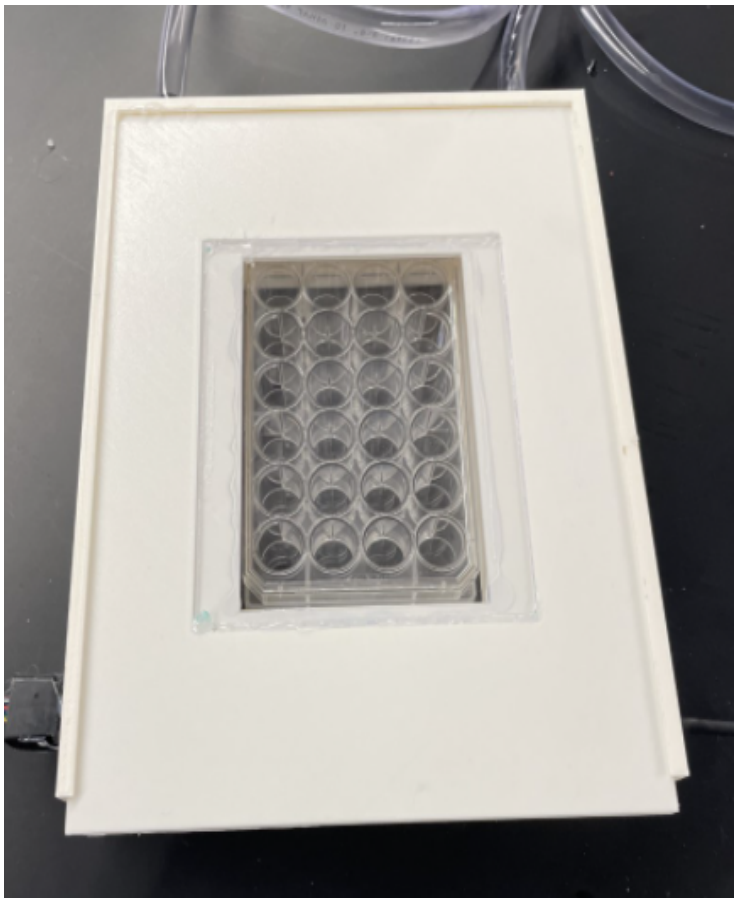


Figure 1: External View of Incubator

Figure 2: Internal View of Incubator

SOLIDWORKS CAD Drawing of the Proposed Cell Culture Incubator

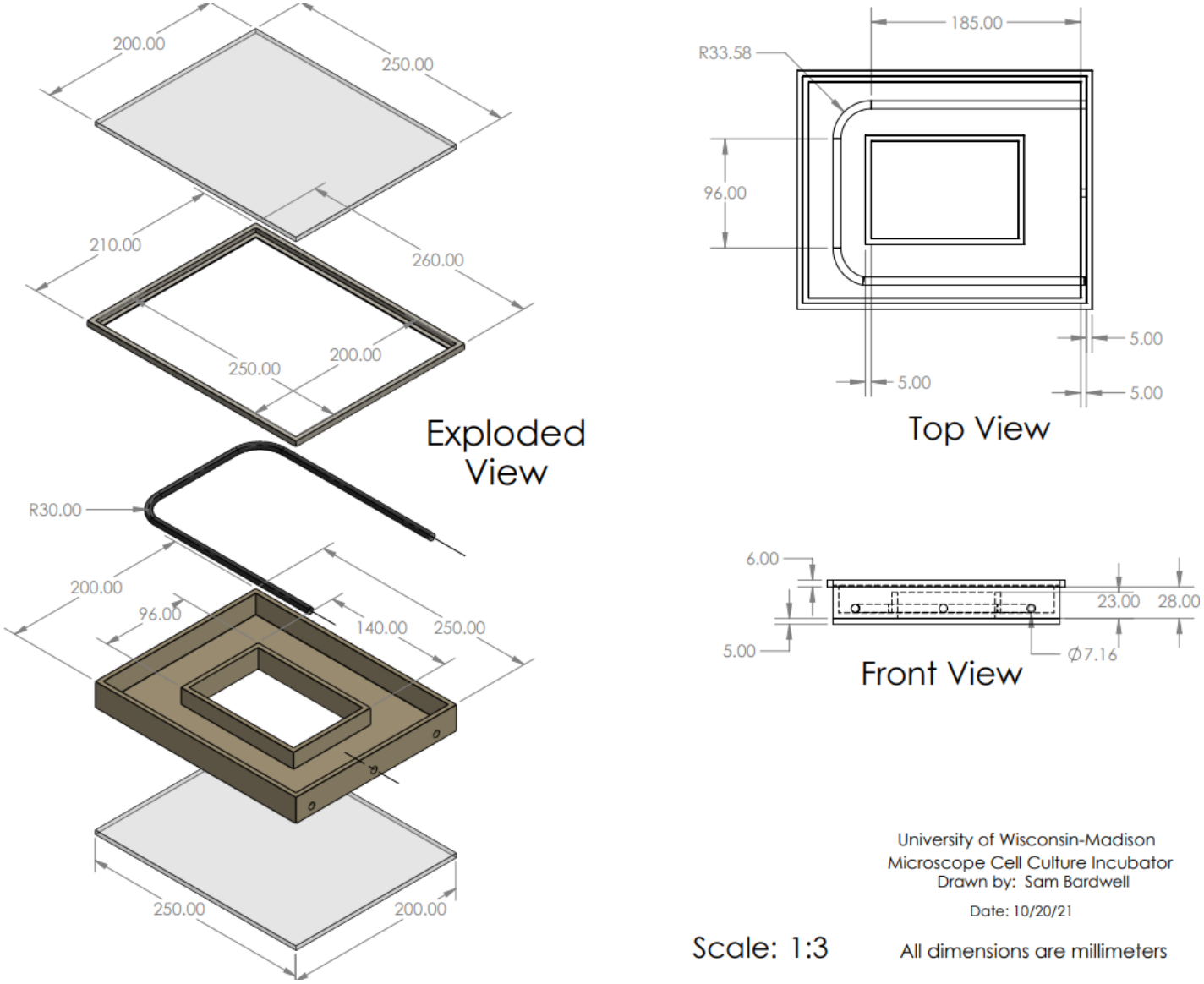
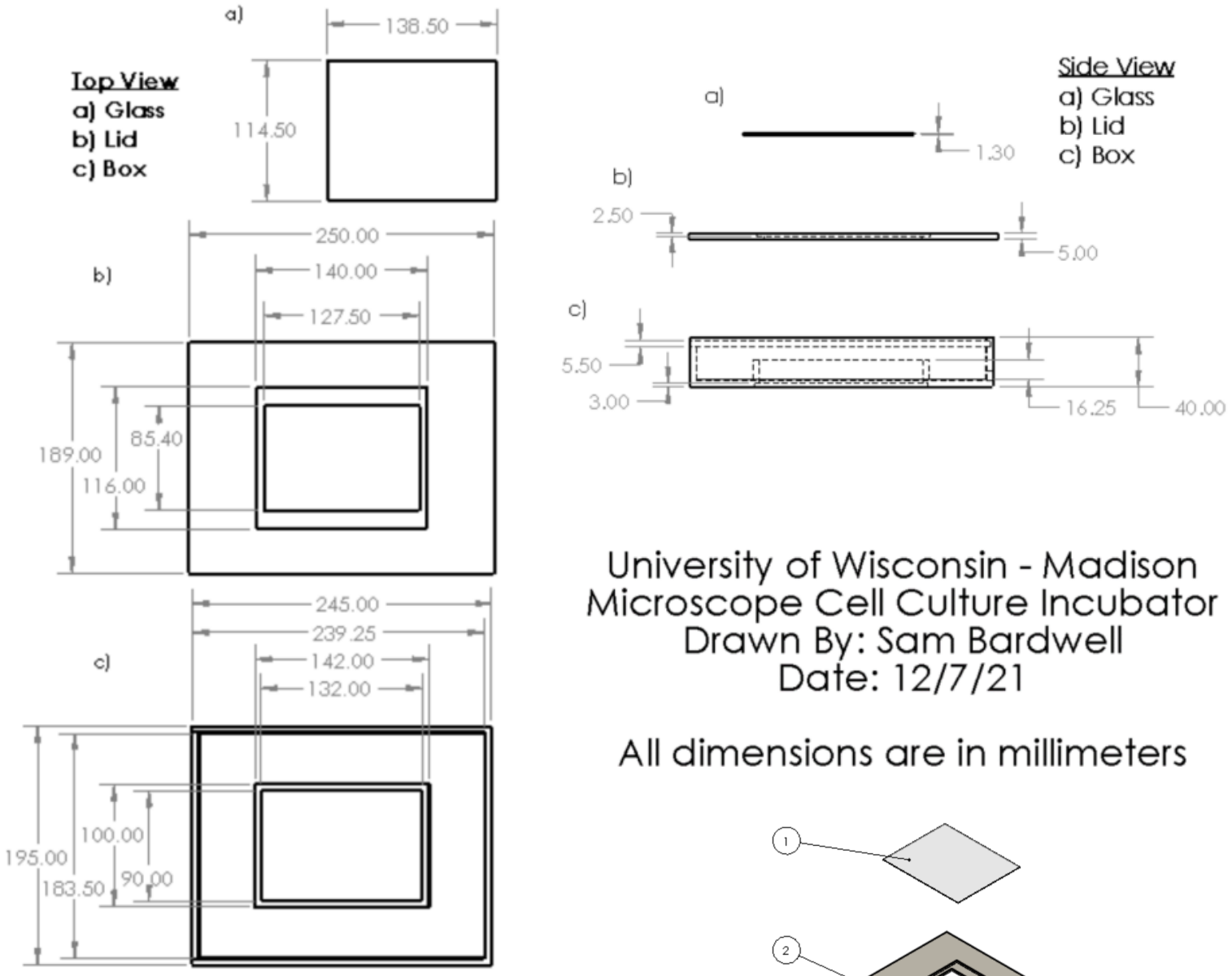


***Figure 3**: SOLIDWORKS Drawing of Design #2*



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 4: 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

Thermistor Circuit Diagram and Code

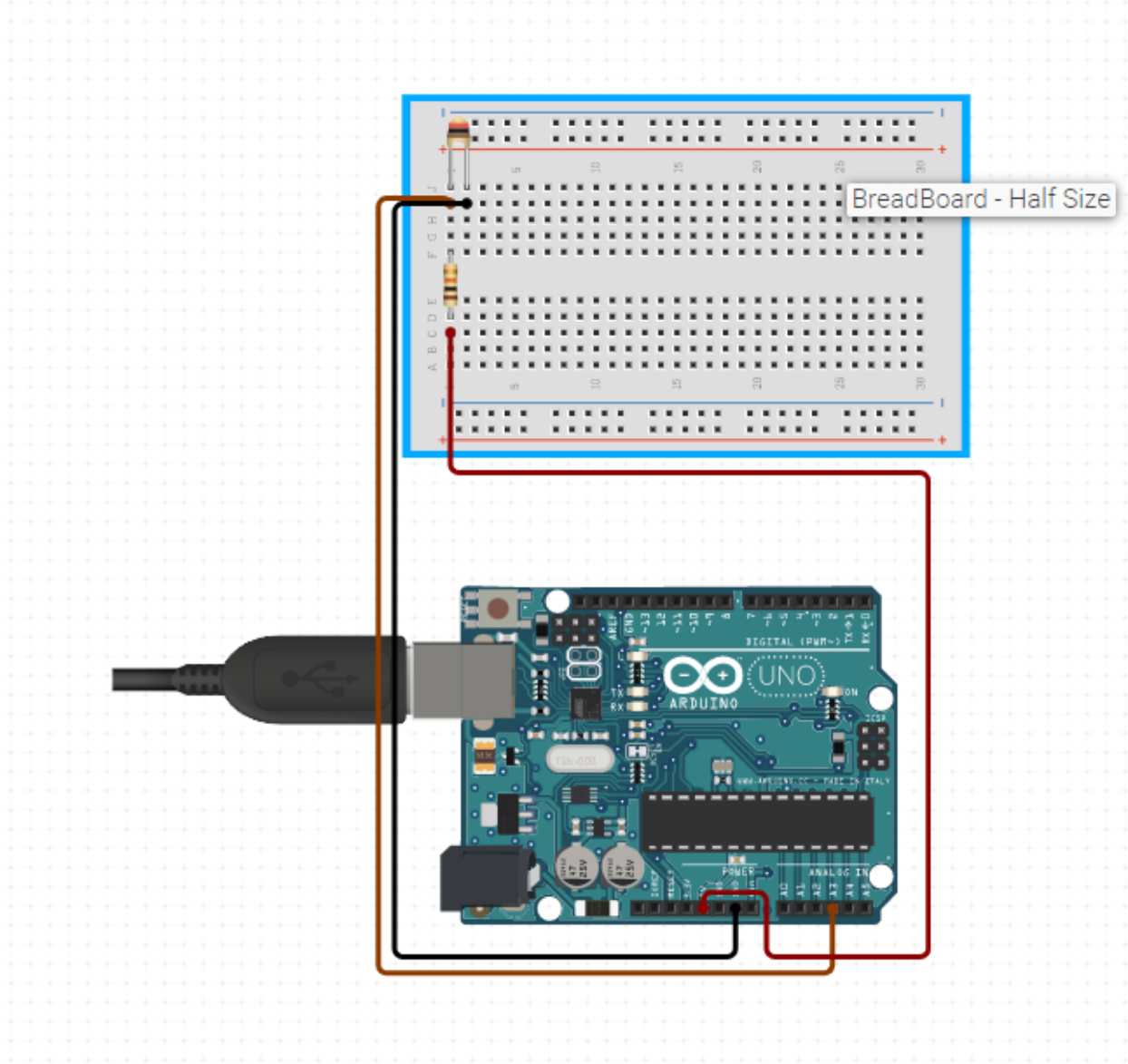


Figure 5: Thermistor Circuit Diagram

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;
double  e_s = 0;
```

```
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;
  e_s = 6.11 *  pow(10, (7.5*Tc / (237.7 + Tc)));

  hum = pow(10, ((20.85 *e_s) - (9.99*pow(log(e_s), 2))/ ((9.99*log(e_s)) - 7.5)    //rel humidity
  Serial.print("Temperature: ");
  Serial.print(Tf);
  Serial.print(" F; ");
  Serial.print(Tc);
  Serial.println(" C");



  delay(500);
}
```

CO_2 Sensor Code and Circuit Diagram

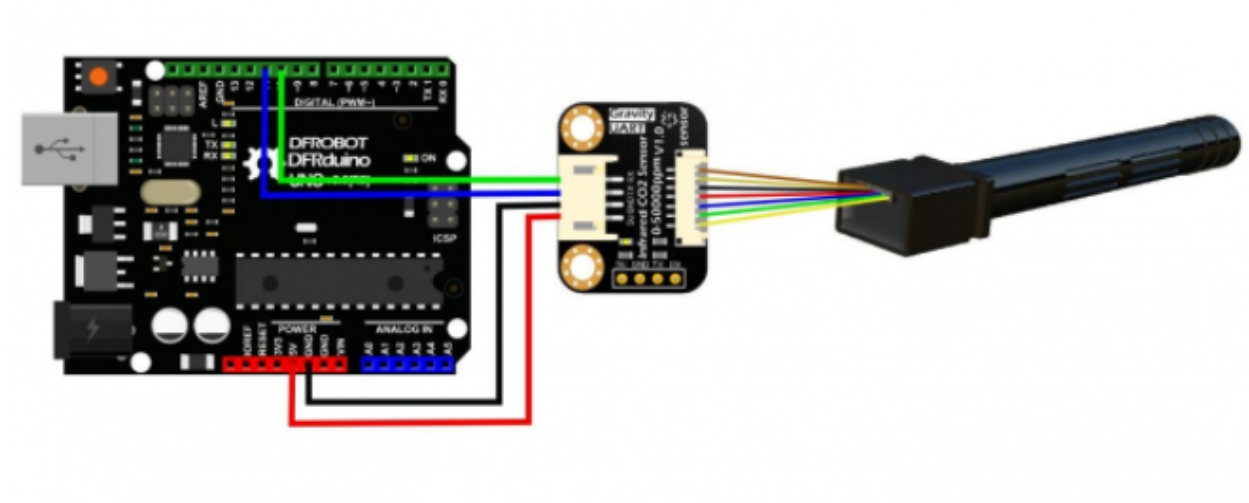


Figure 6: CO_2 Sensor Circuit Diagram [1]

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 C: Testing Protocols

Internal Environment - Temperature and Humidity Sensor Test Protocol

Introduction

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

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. 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. 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:		
2	Test the precision of the Arduino microcontroller at extreme high and low temperatures. Heat a cup of water in a microwave for two minutes. Place the sensor in the cup of hot water 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 Comments:		

	verified.			
3	Set up the incubator for normal use. Set up a digital thermometer within the system.	☐ Verified Comments:		
4	Set up the Arduino sensor and incorporate the breadboard circuits.	☐ Verified Comments:		
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:		
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:		
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:		

Internal Environment - CO_2 Sensor & Feedback System Test Protocol

Introduction

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

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 the CO_2 tank dispenser tube. Allow gas to exit the tank at a low flow rate. Ensure the sensor value readings increase as the sensor exposure to CO_2 gas increases. If this occurs, this step is verified.	☐ Verified Comments:		
2	Similarly, once the CO_2 supply from the tank is turned off, ensure the value readings from the sensor decrease. If this occurs, this step is verified.	☐ Verified Comments:		
3	Set up the incubator for normal use. Record the value read by the fyrite at room conditions in the comments.	☐ Verified Comments:		
4	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	☐ Verified Comments:		

	given by the fyrite, the value given by the CO_2 sensor, and the trial number in the comments.			
5	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:		
6	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:		

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	☐ Verified Comments:		

	record what happened in the 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 the protocol and this 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:
Dates of Test Performance:
Site of Test Performance:

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 resolution test paper between the 2 sheets of High Transparent Lexan Polycarbonate, and place onto the microscope stage.	☐ Verified Comments:		
2	Adjust the optical components of the microscope to best clarity based on personal judgment. Ensure the resolution test paper is centered under the microscope lens. Take an image of what is observed under the microscope.	☐ Verified Comments:		
3	Repeat steps 1-2 without the polycarbonate sheets, but still including the resolution test paper.	☐ Verified Comments:		
4	Have 3 team members, other than the one who completed steps 1-3, complete this step. The smallest element observed without distinct image contrast indicates the approximate resolution limit. Record the group number and	☐ Verified Comments:		

	element number selected by each member in the comments. The team member selecting the resolution limit should assess the image in a blind fashion.			
5	Using the tables and resolution equation provided, calculate the resolution from each team member and the average resolution. Record these numbers in the comments. Higher resolution (lp/mm) is better resolution, and a smaller difference between with the glass and without is better.	☐ Verified Comments:		

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:		
2	Place mammalian cells provided by the client in the incubator. Place the incubator onto the microscope stage.	☐ Verified Comments:		
3	Adjust the optical components of the microscope to best clarity based on personal judgment. Take an image of what is observed under the microscope.	☐ Verified Comments:		
4	Repeat steps 1-3 without the polycarbonate sheets, but still including the cells.	☐ Verified Comments:		
5	Using ImageJ, record the clarity of the images using the microscope focus quality plugin. The images will be divided into regions and assigned a color based on their focus level. Compare these images and their similarity.	☐ Verified Comments:		

Recovery Test Protocol

Introduction

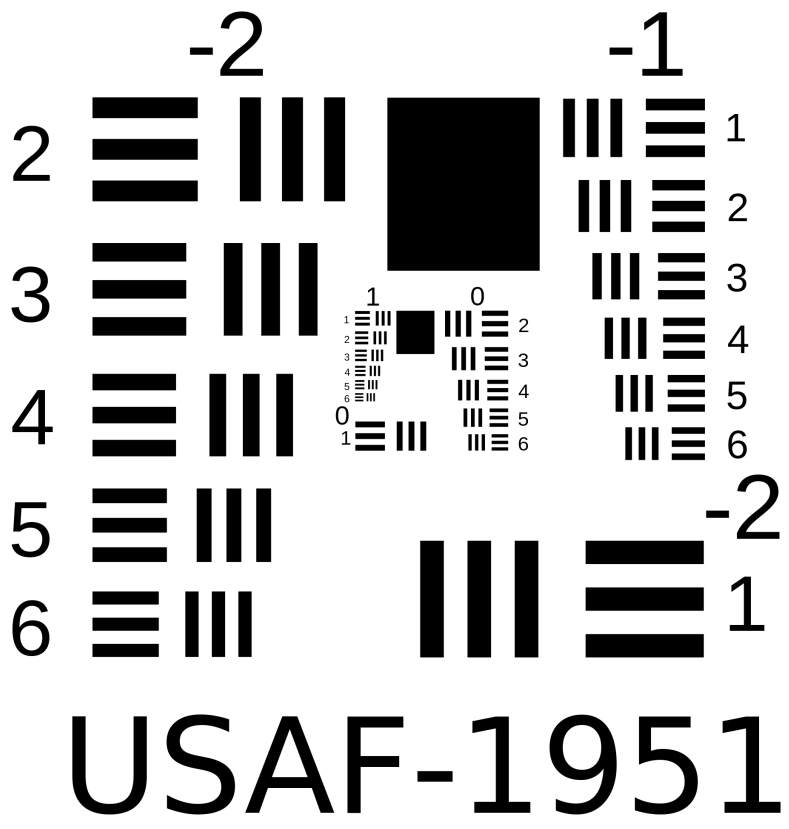
Name of Tester:
Dates of Test Performance:
Site of Test Performance:

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:		

USAF 1951 Resolution Test Chart:



$$\text{Resolution (lp/mm)} = 2^{\text{Group}+(\text{element}-1)/6}$$

Number of Line Pairs / mm in USAF Resolving Power Test Target 1951

	Group Number											
Element	**−2**	**−1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	0.250	0.500	1.00	2.00	4.00	8.00	16.00	32.0	64.0	128.0	256.0	512.0
2	0.281	0.561	1.12	2.24	4.49	8.98	17.96	35.9	71.8	143.7	287.4	574.7
3	0.315	0.630	1.26	2.52	5.04	10.08	20.16	40.3	80.6	161.3	322.5	645.1
4	0.354	0.707	1.41	2.83	5.66	11.31	22.63	45.3	90.5	181.0	362.0	724.1
5	0.397	0.794	1.59	3.17	6.35	12.70	25.40	50.8	101.6	203.2	406.4	812.7
6	0.445	0.891	1.78	3.56	7.13	14.25	28.51	57.0	114.0	228.1	456.1	912.3

Width of 1 line in micrometers in USAF Resolving Power Test Target 1951

	Group Number											
Element	**−2**	**−1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	2000.00	1000.00	500.00	250.00	125.00	62.50	31.25	15.63	7.81	3.91	1.95	0.98
2	1781.80	890.90	445.45	222.72	111.36	55.68	27.84	13.92	6.96	3.48	1.74	0.87
3	1587.40	793.70	396.85	198.43	99.21	49.61	24.80	12.40	6.20	3.10	1.55	0.78
4	1414.21	707.11	353.55	176.78	88.39	44.19	22.10	11.05	5.52	2.76	1.38	0.69
5	1259.92	629.96	314.98	157.49	78.75	39.37	19.69	9.84	4.92	2.46	1.23	0.62
6	1122.46	561.23	280.62	140.31	70.15	35.08	17.54	8.77	4.38	2.19	1.10	0.55